

# The Botanical Review

Interpreting Botanical Progress

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Volume II  
1936

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Published Monthly at  
Lime and Green Streets, Lancaster, Pa.





# The Botanical Review

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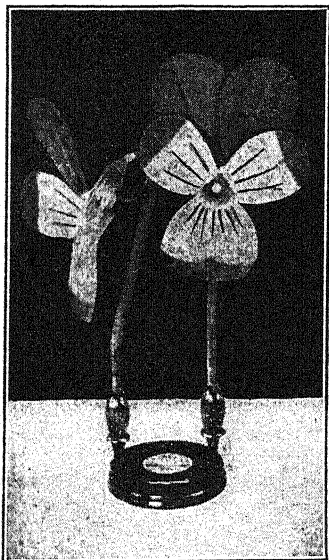
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## ERRATA

Certain egregious errors concerned chiefly with citations to the literature in Part I and Part II of this paper should be corrected as follows:

### PART I

|      |         |           |                |
|------|---------|-----------|----------------|
| Page | 4, line | 22—change | (382) to (332) |
| "    | 4, "    | 27— "     | (64) to (62)   |
| "    | 6, "    | 35— "     | (62) to (64)   |
| "    | 8, "    | 27— "     | (308) to (307) |
| "    | 8, "    | 29— "     | (191) to (291) |
| "    | 12, "   | 39— "     | (276) to (382) |
| "    | 12, "   | 39— "     | (137) to (136) |
| "    | 15, "   | 25— "     | (324) to (349) |
| "    | 16, "   | 30— "     | (303) to (302) |
| "    | 18, "   | 19— "     | (410) to (481) |
| "    | 20, "   | 9— "      | (339) to (341) |
| "    | 23, "   | 38— "     | (409) to (436) |
| "    | 25, "   | 27— "     | (234) to (254) |
| "    | 26, "   | 30—omit   | (410)          |
| "    | 32, "   | 10—change | (493) to (527) |
| "    | 39, "   | 22— "     | (134) to (139) |
| "    | 39, "   | 24— "     | (339) to (340) |
| "    | 41, "   | 26— "     | (388) to (393) |
| "    | 43, "   | 24—omit   | (cytochrome)   |
| "    | 47, "   | 5—change  | (64) to (62)   |
| "    | 47, "   | 9— "      | (64) to (63)   |

### PART II

|      |           |           |  |
|------|-----------|-----------|--|
| Page | 108, line | 22—change | (506) to (577)   |
| "    | 108, "    | 26—omit   | (( <i>cf.</i> 506))  |
| "    | 110, "    | 3—change  | (331) to (329)   |
| "    | 110, "    | 7— "      | (528) to (525)   |
| "    | 110, "    | 20— "     | (tropistic) to (tropic)  |
| "    | 111, "    | 10— "     | (hold) to (holds)  |
| "    | 114, "    | 17—after  | (Popp) insert ((400))  |
| "    | 114, "    | 37—change | (344) to (345)   |
| "    | 118, "    | 8—after   | (Ray) insert (in)  |
| "    | 118, "    | 9—change  | ((1686)) to ((1686)); after<br>(Linnaeus) insert (in); change ((1732)) to ((1732)) |
| "    | 126, line | 17—change | (441) to (414)   |
| "    | 127, "    | 38— "     | (223) to (416)   |
| "    | 128, "    | 33— "     | (198) to (98)  |
| "    | 129, "    | 31— "     | (414) to (408)   |
| "    | 140, "    | 14— "     | (Chialakhian) to (Chailakhian)   |
| "    | 144, "    | 25— "     | (135, 508) to (146, 543)   |
| "    | 148, "    | 22— "     | (91- . 1918.) to (89-204. 1919)  |
| "    | 168, "    | 49—after  | (1935.) insert ((See also: Jour.<br>Amer. Med. Assoc. 93 (2): 1868-1874. 1929.))   |

# THE BOTANICAL REVIEW

VOL. II

JANUARY, 1936

No. 1

## THE RÔLE OF LIGHT IN THE LIFE OF PLANTS

### I. LIGHT AND PHYSIOLOGICAL PROCESSES

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The physiological activities, characteristic growth and differentiation of plant species are brought about by the interaction of all the many factors of inheritance and environment. No one factor taken alone and divorced from all other contributory conditions, such as light, temperature, moisture, minerals, etc., can be considered as being significant in the life of plants. All variables work together and each shares to a greater or lesser extent in the different processes which determine the morphological destiny of the growing plant. Along with other components of the environment, light is known to have profound effects upon the properties of matter and it should be expected, therefore, to have an important influence upon living organisms. A great volume of literature has been written concerning the action of sunlight and artificial light upon plants, so that it is possible to review here only certain selected contributions to the general subject. In the first part of this paper, the rôle of light will be discussed in relation to some of the vital processes, physical properties and chemical constituents of plants,

while the second half of the paper, to appear in a later number, will be devoted to a consideration of the influence of light upon their gross size, form and microscopic structure.

#### RELATION OF LIGHT TO BIOLOGICAL PROCESSES

The source of energy for the plant world is the sun. "Sunlight" comes to the earth's surface in electromagnetic waves varying in length from about 2,900 to approximately 20,000 Ångströms. An Ångström, which is the unit of wave length in common use, is equivalent to  $1 \times 10^{-10}$  meter. Wave lengths of radiation are often measured and recorded also in other terms, such as thousandths ( $\mu$ ) or millionths ( $\mu\mu$  or  $m\mu$ ) of a millimeter. For example, a wave length ( $\lambda$ , Greek letter lambda) of 10,000 Ångström Units ( $\text{\AA}$ . U.) = 1,000 millimicrons,  $m\mu$ ) = 1 micron ( $\mu$ ). The quality, intensity and duration of solar radiation at any point on the earth's surface vary in a definite manner with the time of year, altitude and latitude, and may be modified, further, by atmospheric absorption and scattering and by local obstructions due to topographical features, plant formations, etc. (235, 289). The value of solar radiation at sea level is approximately 1.5 gram calories per square centimeter per minute which corresponds to 10,000 foot candles in terms of illumination (235). Of the total radiation reaching the earth from the sun, about 60 per cent is infra-red, approximately 1 per cent is ultra-violet and the remainder is visible light. Only

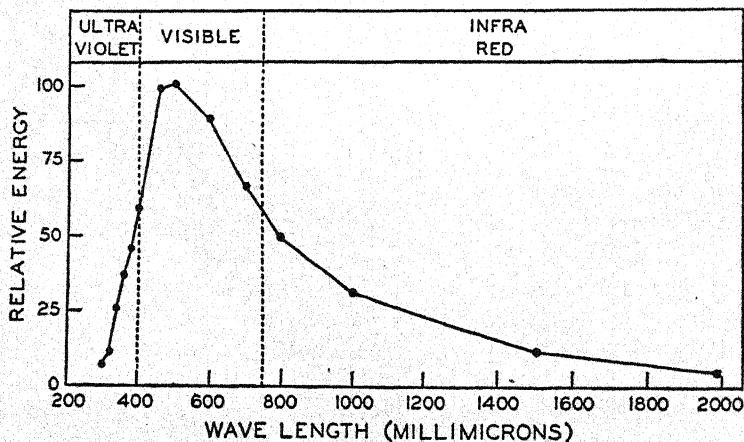


FIGURE 2. Distribution of energy in the ultra-violet, visible and infra-red regions of the solar spectrum at the earth's surface. From Fowle (133).

the visible portion of all the solar radiation, *i.e.*, that fractional part (about 0.75 to 0.4  $\mu$ ) which produces the sensation of light in the human eye, should be regarded strictly as sunlight. However, the word *sunlight* is commonly used to mean solar radiation in a general sense. In recent years, many different kinds of artificial light sources have been used in the growth of plants under various conditions. The wave length and energy distribution of these lamps vary widely, depending upon their temperature and the characteristic emission spectra of the elements employed in their operation (*cf.* 289). Visible radiation is of vast importance in the synthesis of carbohydrates and in its formative effects upon the development of plants. Furthermore, those radiant waves of energy which are shorter (ultra-violet) and longer (infra-red) than the visible rays may be detected and measured by convenient methods, and are known to bring about photochemical and heat effects of considerable importance to plant life.

Researches in the field of photobiology have led to the discovery that the life processes which are influenced by light take place in an orderly fashion, permitting the formulation of laws which precisely describe the biological effects as related to the radiant energy. As evidence of photic stimulation in plants one may observe the products of chemical reactions, tropistic and tactic responses, growth rates, etc. That light must be absorbed in order to produce an effect was first enunciated clearly by Grotthus in 1819 (173). Thus, blue starch iodide is decolorized by yellow light, yellow gold chloride by blue light, and red ferric thiocyanate by green light. Each is attacked by its complementary color, *i.e.*, by the light which it absorbs. The applicability of the Grotthus law has been found true for many biological phenomena, *e.g.*, photosynthesis of carbohydrates by green plants (554, 555), inactivation of enzymes by ultra-violet radiation (153), hemolysis of red blood cells by sunlight (274), etc.

Another law, which was originally proposed by Bunsen and Roscoe (60) for the darkening of silver chloride paper and which is now a well known principle in photochemistry, states that to produce a definite photic effect a constant amount of energy is necessary regardless of its distribution in time. If  $I$  is the intensity and  $t$  the time of its action, then  $It = C$ , where  $C$  is a constant. This law is applicable to simple direct photochemical reactions

where the velocity is proportional to the intensity. As examples of physiological processes which illustrate the Bunsen-Roscoe law, there may be mentioned the phototropic response of the *Avena* coleoptile (31) and the minimum stimulation of *Mya* by light (191). Another well known principle in animal physiology is Talbot's law which states that a reduction in the time of action of light is equivalent visually to a corresponding reduction in its intensity (cf. 192). Whether the intensity is adjusted by rapidly interrupted illumination (as with a rotating sector disc) or by other means, using continuous light, the photosensory value is found to be the same. Rao (421) has reported the validity of this law for the action of light upon seed germination of *Lythrum salicaria*.

Not infrequently, however, some other mathematical relationship is found to exist between the strength of the stimulus and the response. When the magnitudes of the response are plotted against the logarithm of the stimulus and a straight line is obtained, their relationship is frequently referred to (perhaps incorrectly) as the Weber-Fechner law (124, cf. 192). For example, the reaction time in the dark-growth response of *Phycomyces* is proportional to the logarithm of the preceding light intensity (69), and the creeping responses exhibited by many animals are known to be proportional to the logarithm of the intensity (88, 382, 575) of the stimulus. It appears that this law frequently holds true only for stimulation of a certain range and may not apply to relatively low or high intensities. In still other instances none of these simple laws can be applied to more complicated circumstances, as, for example, in the photoleic movements of *Mimosa* (64) and in the light inhibition of seed germination in *Phacelia tanacetifolia* (363), etc., where the responses appear to be hyperbolic functions of the stimuli.

According to the quantum theory of Planck (cf. 592), radiant energy consists of small definite units, the absolute size of which is defined by the equation  $E = h\nu$ , where  $E$  is the quantum (or energy unit),  $\nu$  is the frequency of the wave, and  $h$  is Planck's constant ( $6.5 \times 10^{-27}$  erg/sec). The quanta ( $E$ ) of short waves, where  $\nu$  is greater (since the frequency = velocity/wave length), contain more energy than the quanta of longer wave lengths. The same energy value of violet light of  $\lambda .4 \mu$  contains only half as many quanta as an equivalent energy value of red light of  $\lambda .8 \mu$ . Furthermore, it

has been found that the intensity of the reaction depends, not upon the size, but upon the number of absorbed quanta. One hundred quanta of  $\lambda .4 \mu$  may accomplish the same work as 100 quanta of  $\lambda .8 \mu$ , though the energy of the latter is only half as much. This is the well known law of photochemical equivalence (115) which states that one quantum of radiant energy must be absorbed by each molecule of the photo-reacting material. In other words, reaction rate is proportional to density of absorbed radiation. Not many biological processes have been investigated from the standpoint of quantum activation but it appears that the photochemical equivalence law applies to carbon assimilation (570, *cf.* 222), to photic activation of light sensitive seeds (249), etc. This law, which has formed the basis for a rational development of photochemistry since 1912, would upon proper investigation no doubt be found applicable to many other photosensitive systems in plants.

According to physical theory, matter consists of positive nuclei about which rotate (or oscillate) negative electrons. When the charges are grouped in stable states, which do not radiate, matter exists as atoms and molecules. Atoms are capable of passing from one stable state to another stable state with great rapidity through changes in the electronic orbits. According to Bohr's theory (*cf.* 592), when radiant quanta of a suitable frequency are absorbed by these elementary oscillators the orbits are increased and when radiation is emitted from atoms the orbits are decreased. The phenomena of radiation and absorption may be considered, then, as related to the reversible change in the diameter of the electronic orbits about their nuclei. Absorption of radiant energy by the chemical constituents of plants is dependent upon frequency of oscillation of the electrons of the system, the phase and intensity of radiation, and probably other factors. Once absorbed, the energy may be dissipated as heat to cause a temperature rise, or it may be re-radiated, as in the phenomenon of fluorescence, or chemical reactivity may be brought about by virtue of the active state of the atoms or molecules (*cf.* 185). A formal classification of the different groups of photochemical reactions, as proposed by Weigert (*cf.* 22), would include simple and complex reversible reactions which involve an increase in energy, irreversible coupled reactions with loss of energy in which the products of the photochemical change are used up in other reactions, and irreversible catalytic re-

actions with loss of energy in which the catalyst may exist only during illumination or may remain after the action of light. One of the outstanding characteristics of straight photochemical reactions is their relation to temperature. Their temperature coefficients are very near 1 for  $10^{\circ}$  C. in contrast to ordinary chemical reactions which double or treble for a rise of  $10^{\circ}$  C. (*cf.* 192). An extended discussion of the properties of radiation is not possible here. There are numerous special treatises for fundamental information concerning the theory and technique of light as applied to problems in general physiology (*cf.* 239, 265, 289, 367, 394, 395, 415, 512, 596).

#### PHOTOSYNTHESIS

Through the action of light in the formation of specific chemical substances, such as pigments, hormones, carbohydrates, etc., profound influences are brought to bear, not only upon processes of growth, but also upon processes concerned in the differentiation of specialized cells and organs, and the general course of development in the whole plant. The relation of solar energy to certain chemical constituents and physical conditions which influence the growth habit, cellular structure and reproductive stages in plants deserves special consideration.

One of the most important functions of light in relation to green plants is that concerned with photosynthesis of carbohydrate food which constitutes the supply of energy for growth and development. The nature of the chemical process and the conditions under which photosynthesis takes place in living green plants have been studied by numerous investigators, each attacking a certain aspect of the general problem. The structure of chlorophyll and its characteristic absorption of light energy which drives the mechanism has been elucidated by Willstätter and Stoll (588). The effectiveness of different regions of the spectrum has been worked out on a quantum basis by Warburg and Negelein (570) who reported that five quanta of blue and four of red light were necessary to reduce each molecule of  $\text{CO}_2$ . On a basis of equal energy absorption green light appears to be between the red and blue in its efficiency for photosynthesis (62, 140). Recently, Emerson and Arnold (119), experimenting with effects of intermittent red light, have been able to produce convincing evidence for the existence of two sepa-



rate phases in the process, *i.e.*, the photosensitive phase, activated by light and completed in a small fraction of a second, and the slower chemical phase which may be completed in darkness and which is known as the "Blackman reaction" after the name of its discoverer. With flashing light it has been possible to increase the photosynthesis of sugars per light unit by as much as 400 per cent. The biological aspects of photosynthesis have been dealt with in a long series of investigations by Lubimenko (283) and his associates. This work has been reviewed recently by Priestley (411). The effects of limiting factors in carbon assimilation have been studied by Blackman (33), Lundegardh (291), Van den Honert (207), Van der Paauw (375, 376), etc. Stiles (526) and Spoehr (514) have written monographs reviewing the whole subject in a comprehensive manner. Recent papers by James (222) and Baly (19) treat the dynamical aspects of photosynthesis in a critical fashion, so that extensive discussion of the process seems unnecessary here. The amount, kinds and position of the products of photosynthesis in the plant depend upon the factors concerned in their formation, the enzymes present, the structural pathways for translocation, the relative rates of oxidation (respiration) and reduction ( $\text{CO}_2$  assimilation), etc. The importance of photosynthesis for the present discussion is that food is made available for growth through the action of light upon the chlorophyll apparatus.

#### CHLOROPHYLL FORMATION

The relation of radiation to pigmentation is of very great importance through the necessity of light for formation of pigments, and due to the fact that pigments absorb radiant energy which is essential for physiological activities of plants. Naturally occurring plant pigments, which are found in the protoplasmic structure of the higher plants, are chlorophyll, carotin and xanthophyll. In the sap occur the water soluble pigments, *e.g.*, anthocyanins, flavones, flavonols, etc. In lower plants, such as algae and bacteria, still other pigments are found, as the carotinoid fucoxanthin of the brown algae and the proteinaceous phycoerythrin and phycocyanin of the red and blue-green algae. The last two pigments have been studied extensively by Svedberg (529). The effect of light upon chromogenesis and the possible rôles of diverse types of pigmentation will be discussed briefly.



In the great majority of plants, light is essential for proper development of chlorophyll, though this is not true of seedlings of *Picea*, sporelings of many ferns and mosses (85, 283, 411, *cf.* 291) and some species of unicellular green algae (316). According to Eyster (123), the precursors of chlorophyll may be formed by angiosperm seedlings in darkness and in the presence of light this "prochlorophyll" is changed into green chlorophyll. Temperature affects this dark process but the photochemical change of the precursors to the green pigment is uninfluenced by temperature (284). It is generally considered that in intense light, formation and decomposition of chlorophyll go on simultaneously, and the net concentration of chlorophyll increases with light up to a certain optimum above which the chlorophyll amount is inversely proportional to the intensity within quite wide limits. Lubimenko and Forchel (*cf.* 291) found that the amount of chlorophyll per unit of fresh weight decreased with illumination, while the size of the chloroplasts increased. Holman (205) observed destructive effects of bright light upon the green pigment in living leaves of *Phaseolus*. The relative chlorophyll content differs widely among different plants and along with this variation go differences in the rate of assimilation. According to Lundegardh (291) facultative shade plants seem to possess greater plasticity with respect to chlorophyll concentration than do obligate shade plants. For example, at low intensities, chlorophyll increases with rising light values less rapidly in *Picea* than in *Pinus*. Chloroplasts of shade plants usually are larger and contain a lesser concentration of green pigment than sun plants (308). Shade plants are able to use efficiently small amounts of light and their respiration rates are relatively lower (155) than typical sun plants. Lundegardh (191) has shown that photosynthesis in shade leaves falls off at about one-tenth the intensity of ordinary sunlight, while in sun leaves  $\text{CO}_2$  assimilation may proceed at an increasing rate at five times this intensity. In view of the findings of Emerson (118) and Fleischer (128) that the rate of photosynthesis varies with the concentration of chlorophyll, it may be seen that the quantity of this pigment is extremely important in the economy of green plants.

Several recent papers are of interest in regard to effectiveness of different wave lengths in formation of chlorophyll. Sayre (456) grew seedlings of corn, wheat, oats, sunflower, radish, mustard

and bean under Corning glass filters and observed the progress of pigment formation. Wave lengths longer than 680 m $\mu$  were not effective in the development of chlorophyll but all other regions of the solar spectrum down to 300 m $\mu$  were effective, provided the energy value was sufficiently great. For equal energy values, the effectiveness increased with the wave length to about 680 m $\mu$  and then ended abruptly. However, Shirley (490) found that plants grown under a blue glass, transmitting wave lengths between 374 and 585 m $\mu$  at 10 per cent of the total daylight intensity, often gave a greater concentration of chlorophyll than any other regions of the solar spectrum at the same intensity. The chlorophyll concentration was usually lower under a glass which transmitted only rays longer than 529 m $\mu$ . Colla (82) found that chlorophyll was formed upon exposure of etiolated plants to ultra-violet in the region 330-390 m $\mu$  at an intensity too low for starch formation. Rudolph (447) studied formation of chlorophyll in seedlings of the wax bean grown in darkness and exposed to light for brief intervals, and reported that efficiency for pigment production was proportional to the wave length in the visible spectrum. Meier (315) found that chlorophyll was formed best in algae when the blue-violet region was included in the incident radiation. Guthrie (176) found that the amount of chlorophyll could be diminished by growing plants in continuous light or by removing the blue region from the solar spectrum, and suggested that the red radiation may be injurious when the blue-violet is deficient. Stephan (520) and Johnston (227) both have suggested that high proportions of infra-red radiation may be harmful to the chlorophyll mechanism. The recent paper by Hubert (212) is of interest from the standpoint of chlorophyll decomposition by light, which occurs readily in solution outside the plant.

A few contributions have been made concerning the influence of the daily light period upon chlorophyll development. Some of Garner and Allard's (146) observations are of interest in this connection. Under only 5 hours daily light exposure, sweet potato, soy bean, Irish potato and turnip became etiolated but peanut and *Aster linarifolius* retained their green color. A somewhat longer day but still too short for flower production, caused a deeper shade of green than normal. Poinsettia grown in short days developed the usual red bracts but when transferred to long days vegetative

development was renewed and the red color of the bracts changed to green. Arthur (10) noted that certain plants, particularly tomatoes, were not capable of maintaining their green color under long daily exposures to artificial light. Chailakhian (73) studied chlorophyll development in *Panicum*, *Soja*, *Triticum* and *Pisum* growing under short and long day conditions. Early in the life of the plants, longer days appeared more favorable for chlorophyll development but later, after 3-4 weeks, the shorter day favored pigmentation. Chailakhian supposed that the accumulation and content of chlorophyll in plants growing under natural conditions increases under the influence of the day length and interprets data of Lubimenko for several hundred plants in support of the theory that chlorophyll concentration increases as the distance from the equator decreases.

Considerable interest has been shown in recent years in the phenomenon of albinism in higher plants, especially from the genetic viewpoint. Lebedeff (270) found no difference in the phototropic response nor in the development and growth energy of the leaves of albino and green maize seedlings, and concluded that the physiological determinants were not linked with those for the chlorophyll apparatus. Pollacci (399) claimed that partial albinism in "Mentana" wheat may be overcome by placing the plants in well lighted conditions. Ultra-violet light, even for very short periods, was said to "cure" albinism. Similarly variegated leaves of *Arundo Donax* var. *foliis variegatis* were claimed to lose their albinism when placed in very bright light.

It is usually stated in textbooks that chlorophyll is never found in roots with the exception of certain aerial roots of orchids, but what this fundamental difference is between the root and shoot has not been ascertained. However, chlorophyll has been reported in the roots of barley and several other plants exposed to light (154, 496). Gautheret (154) reported more rapid formation of chlorophyll in barley and *Ipomea purpurea* roots exposed to light when the roots were excised from the plant and supplied with sugar in a culture solution. Powell (405) has reported that in 13 out of 16 species studied, chlorophyll was formed in the roots which were exposed to light.

Reflection of light from the green leaf surface has been measured by Shull (495), and the light absorption of green leaves has been

determined by Willstätter, Tswett, Herlitzka (*cf.* 514), Schanderl and Kaempfert (464), and others. Spohn (515) determined the transmission and reflection of autumn colored leaves. Recently, Seybold (485) reported upon optical properties of albino and normal leaves in a series of articles, showing that in the green leaf about 50 per cent of the incident infra-red is absorbed and about 90 per cent of the solar ultra-violet, while maximum visible light absorption occurs in the long red region at about 670 m $\mu$ . The infra-red absorption spectra of chlorophyll and several other plant pigments have been studied by Starr (516) and the ultra-violet absorption of chlorophyll has been measured by several investigators (275, 100). Lewkowitch (275) found that an alcoholic solution of chlorophyll possessed absorption maxima at 420 m $\mu$  and 325 m $\mu$ . For further information on green pigments of plants the discussions by Lubimenko (283), Dubosc (108), Stiles (526), Spoehr (514), Schertz (468), etc., are helpful.

#### OTHER PIGMENTS

In addition to its action upon chlorophyll, light exerts its influence upon plants through other pigments, as mentioned before. In leaves and fruits of green plants, the yellow-red carotin and xanthophyll are commonly associated with and masked by chlorophyll, while the common red and purple colors of flowers, fruits, etc., are usually due to anthocyanin pigments present in the cell sap. Autumnal coloration of leaves comes about by fading of the chlorophyll, which then unmask the associated pigments (455). Smith and Smith (506) tied black bags around growing tomato fruits and found that no chlorophyll developed in total darkness, but the white fruits gradually changed color to yellow or red (due to lycopin, an isomer of carotin) as they matured. Veselkine and others (558) found that tomato fruits enclosed in black bags remained white with only small amounts of lycopin inside the fruits. Several varieties developed a lower carotinoid content in darkness. Murneek (346) found that when *Cosmos*, *Salvia* and *Soja* were exposed to different photoperiods there were marked differences in the time of sexual development, and that the carotin and xanthophyll increased in plants which had changed from the vegetative to the reproductive state. Rudolph (447) has shown that carotin and xanthophyll are formed less rapidly in red than in blue light.

Norris (366) observed the development of chlorophyll and carotinoid pigments in etiolated plants following irradiation with a 200 watt tungsten lamp for 51 hours. Chlorophyll and xanthophyll increased in a constant ratio but carotin decreased at first and later developed more rapidly than the other two pigments. Euler and Hellstrom (120) found a constant chlorophyll/carotin ratio in etiolated barley seedlings when exposed to light, while the chlorophyll/xanthophyll relationship increased with the age of the seedlings. MacKinney (295) also observed a constant chlorophyll/carotin ratio, which may be taken as further evidence for the interrelationship of these pigments in photosynthesis (*cf.* 19). In a recent book citing 201 references, Lederer (271) has described the properties of carotinoids at length and has concluded that their use to the plant is not yet definitely known.

Formation of anthocyanin pigments under controlled light condition has been studied by Adams (2) who grew several kinds of plants under continuous artificial light supplied by a 700 watt Mazda lamp, and reported normal coloration of flowers as follows: white in wax bean, yellow in tulip, blue in hyacinth, red in the stamens of castor oil bean. Kosaka (251) found that only in the presence of light was the natural pigment formed in the flowers of *Chrysanthemum* and, furthermore, that low temperatures (7–15° C.) favored, while higher temperatures (25–30° C.) inhibited, pigmentation. Kuilman (259) found that a chromogen was produced by a photochemical reaction whose rate was not influenced by temperature. This chromogen was changed to anthocyanin by a dark reaction which was affected by temperature. Mirande (325) described the formation of purple anthocyanin in lily bulb scales under the influence of blue-violet and, to a lesser extent, the red portions of sunlight. This same author (326) found a correlation between oxidase activity and the presence of anthocyanin, as was later found also by Onslow (370). McCrea (312) found that a red pigment developed in the mycelium of *Claviceps purpurea* when exposed to short light rays. Much interesting experimental work has been done recently upon coloration of apples by radiation. Magness (299) found that Jonathan apples were colored by ultra-violet radiation. Fletcher (129) observed that apples bagged in red cellophane on the tree failed to develop the red pigment. Later Pearce and Streeter (276), Arthur (9) and Freytag (137) re-

ported, as a result of careful experiments, that development of red pigmentation in apples is brought about by the blue-violet and the ultra-violet of sunlight. There is considerable evidence indicating that a relatively high sugar content may favor formation of anthocyanin in leaves (172, *cf.* 168).

Since carotinoids show characteristic absorption bands, it is possible that the absorbed energy of certain wave lengths may be converted to chemical use. Other theories have been suggested, *e.g.*, that perhaps these pigments may be concerned with oxygen transference, or that they may control the equilibrium between chlorophyll *a* and chlorophyll *b* (*cf.* 168). Many natural pigments, including glucosides, anthracenes, anthraquinones, etc., are known to fluoresce, but little is known about their uses to the plant. Petri and de Cecco (389) have studied fluorescence in 164 species of plants. Anthocyanin pigments have characteristic absorption bands, one in the middle of the visible spectrum at about  $\lambda$  500 m $\mu$  and another in the ultra-violet (475), the exact position depending upon the nature of the pigment and its solvent (442). Schmucker (472) has studied quantum relations in photosynthesis of green tissues with and without accessory pigments present in the cells, and has found that due to their absorption of the incident light, carotinoids decreased the effectiveness of the blue region by 15 per cent. The presence of flavone and anthocyanin in alpine plants has been ascribed to the action of intense light, particularly the shorter wave lengths, and it has been suggested that these pigments may exercise a protective rôle by screening out the light from deeper lying cells (*cf.* 402). Rosenheim (446) found that less flavone developed in *Edelweiss* when grown at lower altitudes than when grown on the Alps. For further information on plant pigments, the works of Onslow (370), including 879 reference, and Mobius (327), including 300 references, and those of Palmer (379), Gortner (168), Karrer and Helfenstein (232) and others (257, 258) may be consulted.

#### CHROMATIC ADAPTATION

The matter of chromatic adaptation, described by Gaidukov in 1904 (*cf.* 291), has been of considerable interest in connection with attempts to account for zonal distribution of aquatic algae. Wurmser (*cf.* 291) reported that red algae in green light assimilate more

rapidly than green algae under the same conditions, and similar conclusions have since been reached by other workers (333, 552). Harder (184) found that blue races of *Phormidium faveolarum* have their maximum assimilation in red light, while red races assimilate best in blue light. Boresch (43) found that out of 18 species investigated, only four developed a coloration complementary to that of the colored light under which they were grown experimentally. In the complementarily colored species of *Phormidium* and *Microchaete*, red light favored development of blue phycocyanin and green light enhanced formation of the red phycoerythrin. The quality and intensity of light available for submerged water plants vary with depth, color and turbidity of the water (492). Lundegardh (291, 492) states that the matter of adaptation to conditions of low intensity and relatively greater proportions of blue wave lengths in deep water may be determined by either a low compensation point or by absorption and use of complementary radiation. Not only the quality of light but also its intensity is important in maintenance of a favorable  $\text{CO}_2$  balance in the variously pigmented groups of plants. Higher intensities of light are required by green and brown algae than by red algae (552, 330). Lubimenko and Tikhovskaiia (288) are of the opinion that the light spectrum and color of plant plastids are of no primary importance for distribution of marine algae at a depth of 50 meters. Recent papers by Montfort (331) and Seybold (487) show that adaptation of marine species to intensity and wave lengths of light has an influence upon physiological functions. The ability of red algae to absorb a large percentage of blue light explains, in part, their ability to live in relatively deep water.

The relative effectiveness of different regions of the solar spectrum for carbohydrate synthesis is strongly reflected by the ability of plants to grow successfully. The function of wave length in the growth of many different kinds of algae has been studied by Teodoresco (534), Meier (315) and others, and in general it has been found that green forms thrive best when grown under red light in the region of the red absorption band of chlorophyll, though some other groups possessing considerable proportions of other pigments (as the diatoms) may be able to use blue-violet rays to relatively better advantage than can the grass-green algae (534). Purple bacteria can synthesize carbohydrates in darkness, but the rate of



carbohydrate formation is more rapid in the presence of light (556, 443). Green bacterial pigments are, in many respects, similar to the chlorophylls (473). Recent work indicates that absorption of light by the purple pigment has no significance for carbon dioxide assimilation in bacteria (443).

#### PHOTODYNAMIC ACTION

The importance of light-absorbing materials in plants may be emphasized by brief reference to the photodynamic action of dyes which have been experimentally introduced into living tissues. Many years ago Hertel (197) found that ultra-violet radiation of 280 m $\mu$  killed bacteria, while under normal conditions blue and green wave lengths were without effect in this respect. In the presence of dilute eosin, bacteria were killed by green light in the region of the absorption band of eosin, but remained unharmed in blue light which was absorbed, neither by the dye nor by the bacterial cells. The eosin apparently acted as an optical sensitizer much as does chlorophyll which has a very powerful "photodynamic action." Explanation of photic sensitization in plants appears to be similar to the case of the photographic plate. The absorbed dye acts as a light absorbent, and the effect seems to be produced by activation of oxygen or by the oxidized dye product, since oxygen must be present for the phenomena to take place.

The addition of very small amounts of fluorescein dyes to yeast cultures has been found to have little or no effect upon growth of the yeast cells in darkness, but in the presence of light, growth is checked (296). Navez and Rubenstein (324) obtained an increased rate of starch hydrolysis by diastase upon the addition of dyes of the fluorescein series in the presence of light. A very interesting example of photosensitization in relation to growth has been demonstrated by Blum and Scott (38) with wheat roots grown in nutrient solution. After the addition of 1:500,000 erythrosin to nutrient cultures in glass vessels, unilaterally illuminated roots exhibited relatively greater growth rates on the shaded side so that bending occurred toward the light, whereas without the dye these roots were not phototropic. This response was attributed to light-absorption by the dye in a manner similar to that exhibited by the naturally occurring porphyrins in plants which are generally photodynamic.



A photosensitive effect of eosin upon growth of *Vicia faba* roots has been reported by Prescher (406). With appropriate dilute concentrations of dye, root growth and mitosis were hindered by light. Also, Becker (according to Prescher) has found that methylene (1:100,000) is capable of causing abnormal nuclear divisions in direct sunlight but not in darkness. Furthermore, the effect of strong artificial light in overcoming dormancy of seeds and winter buds of woody plants is known to be enhanced by the presence of dilute photocatalysers, *e.g.*, eosin, methylene blue, erythrosin, etc. (355). All this evidence suggests that in the normal course of events, where light exerts an action upon growth, it probably is brought about by absorbing substances (pigments) naturally present in the plant.

Many other examples of optical sensitization are on record in both plants and animals (265, 482). A striking example of photochemical action is the photolysis of red blood cells, where the relative hemolysing efficiency of the solar spectrum coincides with absorption curves of oxyhemoglobin (274). Experiments of Jirovec and Vacha (225) with green and colorless forms of *Euglena gracilis* suggest that oxygen may be contributed to light sensitive reactions through the action of light upon the chlorophyll-photo-synthetic process. Ingestion or injection of certain plant materials are known to render animals sensitive to light (265, 318). An extensive review of the general subject may be found in Blum's (37) paper on "Photodynamic Action."

#### TRANSPIRATION

Loss of water from aerial portions of plants has been given much attention by physiologists. Along with several other factors, intensity of radiation has been found to be of great importance in controlling rate of evaporation from the shoot and, hence, rate of water absorption by the roots (13, 457). Martin (303) has found a linear relationship between transpiration rate in *Helianthus* and light intensity. Depending upon the evaporating power of the air, the fraction of transpiration due to direct effect of radiation varied from 38 to 81 per cent. Other workers have found that water content of plant tissue is lowered and organic matter increased proportional to the square root of light intensity, as the latter is increased over a considerable range (269). The rôle of light in

regulating stomatal apertures through which a large proportion of the evaporation takes place appears to be controlled by variation in the hydrogen-ion concentration of the guard cells which in turn controls their turgor (459). Redington (430) has observed that rapid transpiration may set up a tension on the sap so that water is withdrawn from the differentiating tissue below the meristems. A number of species, such as *Pelargonium*, *Humulus*, *Boehmeria*, etc., when grown under continuous light conditions, exhibited cyclic reduction in leaf area by abscission which was followed immediately by decreased transpiration and increased growth of the stem. It would appear that a method of protection against excessive drying out has been adopted (perhaps fortuitously) by plants. When moisture supply becomes low, the stomata may close even in the light to the extent that both photosynthesis and transpiration may be decreased (76, 474, cf. 492, 48).

Transpiration from tobacco plants in relation to radiant energy in visible and ultra-violet radiation was studied by Arthur and Stewart (13) with temperature and humidity controlled by standard air-conditioning machinery. Within a temperature range of 73°–78° F. the rate of water loss due to visible light greatly exceeded that occasioned by infra-red energy, but at high temperatures of 98°–100° F. the infra-red rate of water loss increased so rapidly that it almost equalled the visible rate. Since the stomata were closed under the infra-red, it is believed that the high rate of transpiration must have been wholly cuticular. It was concluded that transpiration from leaves under high radiation values at high temperatures probably places considerable tension on the water system and thereby diminishes growth rate. That growth is dependent upon a liberal supply of water has been emphasized in the recent work of Loomis (282) where it was found that sunlight and soil and air moisture are important factors contributing to the maintenance of turgor and growth in maize.

#### ABSORPTION AND USE OF SOLUTES

Effects of light in relation to adequate supply of water and nourishing materials to growing regions of plants have received considerable emphasis. Priestley (407) made a special study of morphological and structural features of etiolation in legume seedlings grown in complete darkness. Peculiar suppression of foliar or-

gans, abnormal elongation of the stem and development of a plumular hook were correlated with decrease in superficial cuticle formation and accumulation of fatty and proteinaceous materials in the cell walls of the region intervening between the meristem and the vascular supply. The main features of etiolation were regarded as consequences of altered permeability and decreased diffusion of dissolved food substances to meristematic cells. The author concluded that brief daily exposures to light accelerated migration of these impregnating substances to the surface of the plant and thus increased the aqueous nutrient supply to the apical meristem.

It is not yet sufficiently clear as to how light affects the availability of essential nutrients through modifications of solute absorption, but several contributions may be mentioned. Redington (429) reported that no quantitative relationship existed between the amounts of water and salts absorbed since maize and barley grown in continuous light showed relatively low ash content as compared with the same kinds of plants grown under intermittent light. Scott and Priestley (410) have pointed out that the processes by which water enters the plant are quite distinct from those by which salts enter. Nemec and Gracanin (351, 352) reported that relatively more K was assimilated by barley in violet and red light than in green, while Weissman (587) found that the percent of N, phosphoric acid and K was greater in shade grown barley, rye and wheat than in sun grown plants of the same species. Hoagland, Hibbard, and Davis (202, 203) found that the rate of absorption of certain ions was influenced by light, the uptake of bromine and chlorine by illuminated *Nitella* cells being considerably increased over that of control plants kept in darkness. Concentration of bromine in the cell sap was proportional to the number of hours of daily illumination. Gracanin (169) grew barley in phosphate solutions placed in thermos bottles (to shield the roots from change in temperature) and reported that no differential effect upon P absorption could be shown when the shoots were illuminated or kept in darkness.

In an interesting paper on oxygen relations of plants, Cannon (66) has shown that under certain conditions the partial pressure of  $O_2$  in the stem and probably also in the root increased when

plants were placed in sunlight, which is in accordance with expectations.

Hibbard and Grigsby (198) have studied the effect of Ca and K deficiency along with different daily light exposures upon the growth of *Pisum sativum*. It was found that the light quality and period of illumination had greater effect on the type and quality of growth than did differential salt solutions of K and Ca deficiencies. Absorption of K and Ca was found to be more rapid in the light. Using a full nutrient solution, the dry weight of shoots grown in short daily light periods was about 22 per cent less than that of plants grown in continuous light. However, with calcium deficiency this difference was decreased to about a 2 per cent difference between short day and continuous light regimes. In this connection, the results of Panchard (380) with *Raphanus sativus*, grown to maturity under high and low light intensities, are of interest. It was found that under low light conditions organic synthesis was greatly limited toward maturity while the intake of ash constituents continued, so that the actual per cent of ash content in relation to organic weight was much higher under restricted lighting.

That light, particularly short wave lengths, influences absorption of inorganic nitrogen has been reported by Tottingham and others (547, 548). Absorption of nitrate per gram dry weight of tissue in wheat plants was said to be increased by blue to longer ultra-violet radiation from a carbon arc, when other factors were kept constant. Tottingham, Stephens and Lease (548) tested the effect of ultra-violet upon the absorption of nitrate by growing young wheat plants under Mazda lamps alone, and with added ultra-violet energy supplied by a mercury arc. Both with single nitrates and with a complete nutrient solution, response to increase in the proportion of blue to ultra-violet energy was evidenced by increased nitrate absorption. In another recent paper (546) it has been reported that blue-violet light increased the percentage of protein in the young wheat plant, while pentosans and crude fiber were decreased. Solar ultra-violet radiation was believed to increase the per cent of lipides and of uronic acids. It is difficult to say whether or not all other factors which might have accounted for the observed results were eliminated. Nightingale (359) found that tomatoes, grown in solution without nitrogen,

increased the absolute amount of nitrogen present in the plants, and that a short photoperiod was more favorable than a long one for this increase, even though less distilled water was added to the plants in the short light period.

Nitrogen deficiency is decidedly a limiting factor for growth of the vegetative parts of plants, both directly through its unavailability for the building of protoplasm, chlorophyll, etc., and indirectly through the effect upon  $\text{CO}_2$  assimilation, which is reduced by lack of ample supplies of nitrogen (339). Gile (159) has shown that corn plants grown in nutrient solutions with nitrogen were able to take up in darkness all the nitrogen necessary for growth. If short wave lengths of visible and near visible radiation do increase absorption of certain solutes, the value of this increased uptake for the plant's welfare remains to be proven.

#### PERMEABILITY

The effects of light upon cell membrane permeability have been investigated by Lepeschkin (272, 273) using as a criterion the rate of dye penetration into the leaf cells of *Elodea*. It was found that greater absorption takes place in light of moderate intensity than in darkness "due to the change of permeability of the protoplasm." This increase in absorption can be localized in the illuminated part of a leaf and reaches a maximum increase in about 10 per cent full sunlight. The rays most active in producing the effect were those with a wave length of 320–420  $\text{m}\mu$ , i.e., ultra-violet; less active are the violet, blue, green; least active are the red rays. These results correspond in general with the data of Brooks (57) who found that the amount of 2, -6, dibromo-phenol indophenol penetrating the sap of *Valonia* increased as the wave length decreased. The force of attraction of protoplasm for water has been reported as being decreased by shorter wave lengths of light (329). In a study of the effect of light upon the permeability of *Paramecium* to  $\text{NH}_4\text{OH}$ , Packard (377) found that light exposure increased the permeability. The effectiveness was greater in shorter rays and increased with the duration of the exposure. Permeability of the cells of the *Avena* coleoptile was increased by light in experiments of Brauner (53). Hoffman (204) observed a great effect of light upon the penetration of glycerine into the cells of *Spirogyra*. In contrast with these results, Zycha (600) re-

ported no effect of light upon the permeability of leaf cells exposed to  $\text{KNO}_3$  and  $\text{NaCl}$ . The plasmolysis time for  $\text{KCl}$  and  $\text{CaCl}_2$  with mesophyll cells of *Ranunculus ficaria* has been shown to be short in the dark and long in the light (573). Furthermore, the permeability for sugars in parenchymatous tissues of *Daucus carota* has been shown to decrease by as much as 38 per cent in the light (55). The significance of permeability variations brought about by light has exceedingly interesting implications for the theory of growth-hormone activity.

#### PROTOPLASMIC MOVEMENT

Initiation of protoplasmic streaming in dark adapted cells of *Vallisneria* has been found to be most rapid in red light, less in blue and least in green rays, while infra-red appears to be inactive (480). If protoplasmic streaming is influential in the translocation of solutes (cf. 89), then light certainly must be a factor in the movement of materials in plants. Fitting (127) found also that red rays were most stimulating in causing resumption of streaming in dark adapted cells of *Vallisneria*. Others have found, likewise, that suitable intensities of different wave lengths of visible radiation stimulate protoplasmic movement (23), but that the effect may be retarding or stimulating, depending upon intensity as well as upon wave length (49).

Orientation of chloroplasts under different light conditions is of interest because of the relation of this phenomenon to efficiency of  $\text{CO}_2$  assimilation. Gisl (161) observed that plastids of *Schistostega* collected on the more intensively illuminated part of the inner wall of the lenticular cells of the protonemata. Voerke's (562) observations concerning relative effectiveness of different regions of the spectrum in relation to phototaxis of *Funaria* chloroplasts, show that within the range of intensities employed, the blue, blue-green and yellow-green rays induce the chloroplasts to assume a position next the outer cell wall in a plane so as to expose their optimum surface to the incident light. In red light and in darkness the plastids become arranged along the sides of the cell walls.

#### ASSIMILATION

Any discussion of the chemical constitution of plants in relation to their growth and differentiation would turn naturally to a con-

sideration of fundamental materials, such as carbohydrates, nitrogen and other essential constituents. The dry weight increase of plants is intimately associated with their rates of photosynthesis, and increase in growth rate with increasing light intensity has been found up to 50 or 100 per cent of ordinary sunlight (490).

For optimum growth of any green plant under a given set of conditions with respect to temperature, mineral nutrition, water supply, etc., there is a certain light exposure of optimum intensity, quality and duration. The quantitative relationships between

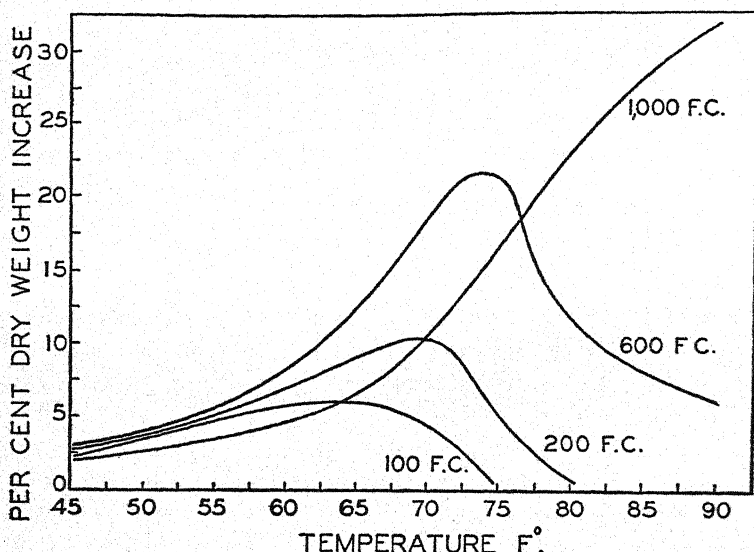


FIGURE b. The assimilation rate in young tomato plants in relation to light and temperature. Under a low light intensity of 100 foot candles, the relative increase in dry weight reached a maximum rate at about 65° F. With increasing intensity, the maximum was shifted toward the higher temperatures. Under bright light of 1000 foot candles, even a high temperature of 90° F. was favorable for assimilation. After Bolas (40).

radiation and temperature and plant growth have been worked out in an interesting fashion by Bolas (40) in experiments with the tomato. Separate groups of plants were grown in glass houses under several different light intensities varying up to 1000 f. c. For each intensity there were also several different temperature values ranging from 45° F. to 90° F. The per cent increase in dry weight in a 7-hour period showed an optimum value at higher light intensities as the temperature increased. At 60° F., assimi-



tion reached a maximum at about 100 f. c. light intensity, and decreased markedly with increased intensities at the same temperature. However, at 85° F. assimilation increased with increasing light intensity up to a maximum near 1000 f. c.

Gilbert (157) investigated the effect of different day lengths and temperatures on the growth of six species of plants. Specimens of *Xanthium pennsylvanicum* were grown in 11 hours and 14 hours of light daily, both in high temperatures (60–90° F.) and low temperatures (40–70° F.). Under both temperature conditions the longer daily exposures permitted much longer periods of vegetative growth and greater final height before reproductive processes terminated the experiment. The greatest vegetative development occurred under long day and low temperature conditions, and the author concluded that the action of the light period on the plant is modified by humidity, nutrient supply, and temperature in accordance with Blackman's theory (modified perhaps, cf. 222) of limiting factors; the latter states that the pace of a process is set by the factor in minimum (33). Eaton (109) found that soy beans exposed to a day length of 13 hours and subjected to different temperatures at night, e.g., 90°, 65° and 50° F., gave the greatest average height and total dry weight at the lowest temperature.

For the building of protoplasm, protein synthesis is of prime importance, and experimental evidence indicates that all living cells have the ability to form protein, provided that supplies of carbohydrates and suitable nitrogenous compounds and other essential constituents are available. That light is not directly essential to this process has been demonstrated conclusively (338, 111, 361). Indirect effects of light upon the processes leading to protoplasmic synthesis are important nevertheless, not alone through the supply of carbohydrates by photosynthesis (360, 362) but also through increased uptake of nitrate from the nutrient solution during exposure to shorter wave lengths of solar radiation (547).

There is also some evidence to show that the quantitative use which plants can make of carbohydrates and inorganic nitrogen may be controlled by daily light exposure (359, 546). If light is insufficient for rapid synthesis of carbohydrate foods, nitrogen cannot be used effectively (409). Under certain conditions proteins are respired as the source of energy (362), especially when



carbohydrates and fats are diminished by conditions unfavorable for photosynthesis. Kishi and Yokota (238) have observed that proteins were decomposed when mulberry plants were shaded.

The total and relative proportions of the different kinds of carbohydrates occurring in plants have been studied in relation to light environment by Garner, Bacon and Allard (150), Deats (96), Hurd-Karrer (216), Arthur, Guthrie, and Newell (12), and many others. Garner, Bacon and Allard (150) found that elongation of the stem of summer radish resulting from long daily exposures to light was associated with an increased content of reducing sugars, particularly in the upper portion of the stem. Changes in the form of carbohydrate content and in the degree of hydration of the plant tissues were among the earliest observable effects of change in the length of day to which plants were exposed. Transfer of *Cosmos* from a long to a short day resulted in material increase in reducing sugar in the upper portion of the stem within 48 hours after the transfer had been made. Two days later the increase in sugar content was in the form of polysaccharide accompanied by a slight decrease in the water content of the plant. Twelve days later the increased content of sugar was again in the form of monosaccharide, flower buds had appeared and the water content of the tissues had increased. In Biloxi soy bean exposed to natural length of day in late summer a slight increase in reducing sugar was found in the leaves at about the time of flower bud formation, followed by a decrease at the time open blossoms appeared. A marked increase in reducing sugar and a small increase in soluble nitrogen was observed 15 days later during rapid development of the fruit.

Deats (96) found that cell sap concentration in the leaves was greatest in plants exposed to a long day, but it was more concentrated in the fruit of tomato which had grown in a short day. Hurd-Karrer (216) found that in young wheat plants the glucose, total sugar, acid-hydrolyzable material and total carbohydrate increased under long day conditions. Glucose content of the culms was 2 to 3 times that found in the leaves, though there was little difference in the pH of culm and leaf. Other investigations have shown that interception of sunlight may bring about increase in the per cent of water and of non-protein nitrogen, and decrease in the per cent of dry matter, the pH and the soluble carbohydrate

(238). Nitrogen fixation by symbiotic bacteria in soy beans is known to be enhanced by regulating light intensity to a value which gives a suitable carbohydrate content (371).

Arthur, Guthrie and Newell (12) have shown that various values for the carbohydrate fractions may be obtained, depending upon when plants are sampled in relation to their light exposure period. In radish the total carbohydrate varied from 7.49 to 34.73 per cent among plants which showed flowering response, while a range of 8.95 to 21.23 per cent was found for those which did not flower. Flowering was initiated by illuminating the plants for 8 hours each night with 170 f. c. without any resultant accumulation of carbohydrates. By using an intensity of 700 f. c. for 6 hours each night, flowering was induced as well as an accumulation of carbohydrates. Also nitrogen supply over a considerable range was controlled in sand cultures without effect upon the flowering stage. The authors concluded that in radish (and also in other plants), flowering was quite independent of carbohydrate-nitrogen relations and depended only upon day length. It was also shown that the per cent of simple carbohydrates in cabbage was doubled on a 19 hour day as compared to a 5 hour day.

#### CARBOHYDRATE/NITROGEN RATIO

In the past few years, considerable attention has been given to the relative proportion of carbohydrates and nitrogenous compounds in plants and their influence upon various developmental phenomena. In 1916 Fisher (126) reported that the vegetative condition in plants is characterized by a low carbohydrate/nitrogen ratio, while the reproductive stage is characterized by a high ratio of these constituents. Kraus and Kraybill (234) investigated vegetative growth and reproduction in the tomato in relation to available carbohydrate and nitrogen supplies, and found that the ratio of carbohydrates to nitrogen was an important determining factor for growth and differentiation. Four different conditions of C/N and the associated developmental characteristics were described: (1) With an abundance of water and nitrogen and low carbohydrate supply, the plant was weakly vegetative and non-fruitful. (2) With little nitrogen and abundant carbohydrate both vegetation and fruitfulness were suppressed. (3) With abundant nitrogen and medium carbohydrate supply, vigorous vegetative

growth ensued but the plant remained sterile. (4) Starting from conditions favorable for vegetative growth, a decrease in nitrogen with a slight increase in the carbohydrate reserve, caused less vigorous growth and induced fruitfulness. Investigations of Klebs (240, 241) concerning effects of light intensity, temperature and soil nutrients in the development of *Sempervivum*, pointed to the same general conclusion, *i.e.*, that not the absolute values but the relationship between the several factors is of consequence. Klebs found that vegetation and reproduction could be controlled by altering the environment in a suitable manner. Plants ready to flower could be reversed back to the vegetative condition and caused to form vegetative rosettes on the elongated axes by reducing the light, keeping the temperature at about 26° C. and supplying plenty of fertilizer. Very strong light caused flowering without formation of an elongated axis.

Many other investigations have since given support to this general principle of C/N balance (358, 359, 362). Miss Hicks (200) followed the distribution of carbon and nitrogen in different organs of the wheat plant through different periods of its life history, and reported that successive stages of development are initiated by critical values of the carbon/nitrogen ratio, that this ratio increased throughout the vegetative period and, when sufficiently high, flowering occurred. A low ratio of carbohydrates/nitrogenous materials appeared to be characteristic of growing points. Conditions favorable for flower initiation may not be favorable for fruit formation (362) because, following fertilization, the young fruit acquires the chemical character of a growing point (200, 342) and absorbs proportionately greater amounts of nitrogen (*cf.* 210). That a high C/N ratio favors initiation of roots in cuttings was pointed out by Reid (433, 434, 410, 438). Also Schrader (476) found that relatively greater proportions of carbohydrates favored rooting in tomato cuttings. Furthermore, it has been found that when cuttings of *Salix viminalis* are planted, shoots grow from the area of lowest C/N ratio and roots from the regions relatively higher in carbohydrates (199). The interrelationship of carbon and nitrogen in growth of germinating seeds with different kinds of stored food has been worked out with great care (435, 436, 437).

As Kraus (253) has pointed out, the changed or changing morphological expression is the external evidence of changed or

changing chemical composition. The same author has emphasized that the conditions which determine meristematic differentiation are quite different from those accompanying further development of the various parts in question. The conditions for flowering may not be those for fruit setting and development. Nitrogen is not all-important as a factor in the growth/differentiation balance but other stuffs, such as sulphur, phosphorus, potassium, etc., the water supply, light and temperature are all significant.

The discovery of photoperiodism came at about the same time as did the announcement of the C/N principle, and in the years immediately following, numerous efforts were made to interpret the effects of light upon plants in terms of its influence upon the relative proportions of the building materials necessary for growth and development. Nightingale (358, 359) grew many kinds of plants under long and short daily exposures to light and with varied supplies of nitrates, and obtained results which agreed in general with the C/N relations previously outlined by Kraus and Kraybill (254). An important point of distinction was found by Nightingale in *Salvia*, buckwheat, soy bean and radish grown in a short 7-hour daily light period wherein plants were not able to fully utilize their carbohydrates, apparently through inability to synthesize the supplied nitrate to other forms of nitrogen. When these high carbohydrate short-day *Salvia* plants were transferred to long day conditions, the ensuing rapid growth was accompanied by more rapid nitrate assimilation and greater accumulation of organic nitrogen. The author expressed the opinion that the significant ratio in growth is that of carbohydrate/insoluble nitrogen, since accumulated nitrate did not affect flowering. It should be pointed out that the *Salvia* plants grown during a day of 7 hours and with full daylight supplemented at night by 6 hours of weak artificial light, showed no significant differences in the per cent of carbohydrate or total nitrogen. Apparently, the additional energy of the longer days made but little contribution to the photosynthesis beyond that found in the 7-hour day. The point to be emphasized is that flowering occurred in the short daily exposures, while in the long days the reproductive stage was not attained. In forcing plants with supplementary artificial illumination, Oden (368) found that the ratio between carbohydrates and proteins increased with an increasing amount of the longer wave lengths of

light. Gilbert (157) grew *Xanthium* in long and in short days, and found that C/N ratio ascended as the plants approached maturity. Hurd-Karrer (216) observed that in young wheat plants, sampled at the age of tillering (branching from the base), the total carbohydrates were highest and the per cent of nitrogen was lowest in the leaves when the plants were grown in long day conditions which accelerated culm elongation and flowering. The lowest carbohydrate and highest nitrogen percentages were associated with a short day exposure, which retarded heading and gave large vegetative plants, with reduced yield of grain or total sterility. Correlations between the carbohydrate/nitrogen ratio and fruitfulness were found only in the analyses made on the culms, and not in those made on the leaves.

In the opinion of many investigators, the magnitude of the carbohydrate/nitrogen ratio is probably not the primary cause of the growth/differentiation balance. As a result of studies in the relation of carbohydrates and nitrogen in the flowering behavior of apple spurs, Harvey and Murneek (189, cf. also 209, 210) concluded that the C/N ratio is important as an indication of possible limiting situations, but that it should not be given a causal relationship in bringing about a particular formative change in the plant. Tincker (542) found that *Helianthus tuberosus*, grown in a reduced light period was unable to use all the carbohydrates for shoot growth and stored the excess food in tubers. In *Phaseolus multiflorus*, grown under short day conditions, the food was stored also in the thickened roots. A relatively high C/N ratio was correlated with earlier flowering, and the author concluded that the length of day controlled the utilization of the photosynthetic products and the rate of stem elongation, and thus influenced the C/N ratio which appeared as a result rather than as a cause of the morphogenetic trend. It is of interest in this connection to note that no difference has been found in the course of photosynthesis in millet grown under 9 and 18 hours of light daily (530). The results of Hibbard and Grigsby (198), who reported that the per cent of nitrate in *Pisum* increased in short photoperiods, appear to lend support to the theory that light exerts some control over utilization of food by the plant. Jaccard and Jagg (221) have found that plants grown under continuous light show less mean CO<sub>2</sub> assimilation per hour and per unit leaf area than plants grown under periodic light conditions.

Hopkins (211) grew soy beans under different lengths of day, different intensities of light and in different supplies of nitrogen. The short-day plants accumulated much starch and were also high in nitrogen, whether nitrate was added or not. In long day conditions, the plants were lower in all forms of nitrogen and in carbohydrates. Shaded plants, with and without the addition of nitrate, were generally lower in carbohydrates and higher in all forms of nitrogen than unshaded plants. The weight of nodules expressed as per cent of the total plant weight was decreased by high nitrate, short days and shading. Kraybill (256) found that shaded apple and peach trees contained more soluble and insoluble nitrogen and less sugars, starch and hydrolyzable material than unshaded trees.

Recently, Müller and Larsen (341), growing plants in N and K deficiencies over a range of light intensity, have found that nitrogen deficiency exerted a depressing effect upon the assimilation rate through some protoplasmic factor, and at high light intensity growth in area of the leaves was reduced.

Purvis (416) grew cereal plants under different day lengths and with varying nitrate supply and observed that nitrogen starvation increased the total sugar content where flowering occurred and also where it failed. Since short days which reduced the assimilating period by 40 per cent had but little effect on the sugar content, it was concluded that the effect of short days on flowering can not be exercised through concentration of sugars. Under conditions of nitrogen starvation, flower primordia appeared and flowering took place at the normal time in spite of reduced vegetative growth. An increasing amount of reducing sugars was apparent just before flower emergence in the experimental plants, but since this was preceded by flower differentiation, it was regarded as a result rather than as a cause of the onset of the reproductive phase. It was concluded, therefore, that the C/N ratio bears no causal relationship to the ability of a plant to differentiate flower initials or to produce flowers. The great range of C/N accompanying the initiation of flowering in barley and millet convinced Borodina (44) that some other immediate cause was active in the change from the vegetative to the reproductive stage. Other workers have emphasized that the accumulation of insoluble carbohydrates is a better measure of the past history of the plant than of its present or future responses (*cf.* 593, 404).

Results of thorough investigations carried out by Arthur, Guthrie and Newell (12) yield further evidence along this line. Many kinds of plants were grown under different controlled conditions of temperature, humidity, light intensity, light period and CO<sub>2</sub> supply. Chemical analyses were made at various times during the period of growth. Depending upon when the plants were sampled in relation to their light exposure period, various values for carbohydrate content were obtained, but the total nitrogen remained nearly constant. Tomato plants kept in darkness for 17 hours lost considerable of their sucrose and dextrose, and after about 40 hours these fractions decreased to about one third the original content. In general, percentages of carbohydrate and nitrogen could be changed by varying the light intensity, length of day, or in some plants by changing the nutrient supply in sand cultures. The range of variation of the carbohydrate and nitrogen fractions varied among the species. Over a 5 to 24 hour range of daily light exposure, *Salvia* was able to maintain a comparatively narrow fluctuation in carbohydrates and nitrogen, but under the same conditions many other plants showed large variations. The authors stated that "no relation was found between carbohydrate and nitrogen content and flowering in either long-day plants such as radish and lettuce, or in *Salvia*, a short-day plant, or in buckwheat, an everblooming type." The available information suggests that photoperiodic responses are not governed by rate of carbon assimilation, but probably are due to other photochemical reactions which can be brought about by relatively low intensities of red light, and the effects depend not so much upon the quantity of radiation as upon the actual length of the exposure period. (426).

#### INORGANIC ELEMENTS

The importance of certain constituents other than nitrogen and carbohydrates in light relations of plants has received attention recently. Borodina (44) observed that phosphorus deficiency delayed, while lack of nitrogen hastened, the earing stage in barley, a long-day plant. Lack of potassium under conditions of a long day (18 hours) delayed the earing stage; with a short day the plants perished without having reached this stage. Apparently the exclusion of essential nutrients from the culture solution makes itself felt more strongly with a short day. In the short-day plant,



millet, lack of phosphorus depressed the plant strongly and delayed formation of panicles, but a deficiency of nitrogen or potassium was without effect on flowering. Eidelman (113) found an increase in photosynthesis with phosphorus present, as compared with controls lacking this element, and in a later paper (114) pointed out the interrelationship between temperature, phosphorus nutrition and photoperiodic response. The presence of phosphoric acid and certain concentrations of sugar in the cell sap has been considered important in the flowering of mountain rice (278). Nemec and Gracanin (352) grew rye under colored glasses (energy not equated) and reported little difference in the phosphoric acid uptake, but found that less potassium was taken up from the culture in green, and more in violet and red light than in sunlight. Matskov(304) could find no direct correlation between intensity of photosynthesis and formation of dry matter, but ample potassium promoted the translocation of assimilated matter from leaves to roots. Potassium has been reported to have some influence also upon the development of chlorophyll and the height of plants grown in various light intensities (456).

Gassner and Goeze (152) have found that when light duration (10 hours or more) is not limiting in the life of young barley plants, a direct relationship between nitrogen supply and assimilation, transpiration and chlorophyll content (number of chloroplasts) can be demonstrated. With short days (3 hours), where light is a limiting factor, no response to nitrogen is apparent. Unlike the nitrogen effect, it was possible to demonstrate even in short days an optimum K supply below which assimilation fell off rapidly and above which it decreased gradually. Since K and N are important for their effects upon protein synthesis, it is necessary to bear in mind the influence of nutritional factors in any interpretation of the rôle of light in physiological processes.

Pfeiffer (390) studied microchemically the effect of intensity and duration of light upon calcium, magnesium, phosphate, nitrate, proteins and carbohydrates in tomato, buckwheat and four-o'clock. In all plants with short light exposures there were usually low carbohydrates and low protein reserves and in longer light periods there was an increase in carbohydrate without a proportionately increased elaboration of protein, perhaps due to a limited nitrate supply. An ample supply of nitrogen is important also for the



highest photosynthetic efficiency (340). Phosphate and magnesium were usually lower under shorter exposures, due possibly to their proportionately greater utilization in protein synthesis and tissue formation. Tincker and Darbishire (543) grew tuber-forming plants under short-day conditions, which enhanced the storage of food and potassium in underground organs. When potassium was rendered deficient, there was less translocation of dry matter into the storage organs, but the conclusion that K is necessary for translocation does not seem to be well founded (*cf.* 523). Street (493) analyzed field peas grown in nutrient solution, and reported that light exposures of 10 hours daily produced plants high in potassium and low in calcium and magnesium. However, exposures of 17 hours daily resulted in plants markedly low in potassium and usually very high in calcium and magnesium.

Investigations of etiolated plants have shown that magnesium content is decreased (27) in dark grown plants. A chemical study of expressed juice from etiolated wheat seedlings indicated that primary and secondary phosphate form the buffer action of the tissues, and asparagin appears to be the substance responsible for the peculiar inflection in the etiolation curve (214). Priestley (408) found it impossible to plasmolyze the differentiated cortical cells of plants grown completely in the dark, due to the presence of lipoidal substances causing adherence of the protoplasts to the cell walls, but after very brief daily light exposures these cells plasmolyzed readily.

Warrington (571) experimented with boron deficiency in plants grown under different lengths of day, and found that boron deficiency symptoms were less pronounced under short day than under long day conditions, and also that in the absence of boron the photoperiodic responses of long and short day plants were less evident.

#### ACIDITY, STOMATAL MOVEMENTS, ETC.

Fluctuation of organic acids with alternating light and darkness in relation to colloidal hydration and growth has been discussed by Long (280), Tolmachov (544) and others. Eisenmenger (116) found that when actively growing tobacco plants were placed in darkness with and without a supply of nitrogen for a period of 11 days, the nitrate and amino acids accumulated

in the darkened plants. Garner, Bacon and Allard (150) investigated hydrogen-ion concentration of cell sap in different plants grown under different photoperiods. In the case of short-day plants grown under long daily periods of light, upward elongation of the vegetative stem was associated with progressive increase in active acidity, particularly in the region of the growing point which became more acid than the lower regions. Under short day exposure, the upper portions of the plant were less acid than the lower. Abrupt transfer from a long to a short day caused a sudden temporary decrease in acidity in the region of the growing point, which change was believed to indicate a transition from the vegetative to the flowering condition. Increased acidity was obtained also when the short daylight period of winter was prolonged by use of electric light of low intensity. In the case of long day plants, exposure to a short day tended to inhibit stem elongation and to keep acidity to a low level, while exposure to a long day resulted in elongation of the axis and flowering, which form of development was associated with general increase in acidity. However, there were considerable differences in distribution of active acidity in various parts of different kinds of plants. Loehwing (277) observed a diurnal pH cycle correlated with variations of light. Strong insolation depressed the sap acidity in *Triticum* to the extent of causing precipitation and ultimate unavailability of iron, leading to chlorosis.

That light brings about changes in the active acidity of the stomatal apparatus in the leaves of higher plants and in this manner influences stomatal opening has been discussed in recent botanical literature (385, 458, 459, 497, 503). Scarth (459) reported that in the presence of light acidity was lessened in the green guard cells, their turgor was increased and the stoma opened, while in darkness a reversal of the conditions took place. Pekarek's (385) experiments with vital staining in the stomata of *Rumex* supported this view. Sayre (457) has shown that the long ultra-violet and visible light is effective in stomatal movement, while the infra-red region of the solar spectrum is apparently without effect. Sierp (497) reported that the quantum theory did not apply to photic activation of stomata, the yellow, green and blue wave length regions being about equally effective in opening stomata, while red light was less effective and infra-red inactive. However,

Paetz (378) reported that stomatal movement was strongest in red light, the degree of response of the guard cells corresponding with the strength of the absorption bands of chlorophyll. Since the rate of photosynthesis follows closely the size of the stomatal aperture (155), it can be readily seen how important is the regulation of turgor in the guard cells by light and moisture supply.

#### PHOTOPERIODIC STIMULATION

The influence of the duration of light exposure upon vegetative growth, reproduction, food storage, etc., in plants has become a favorite topic for research since the general principles were pointed out by Garner and Allard in 1920 (145). Certain experimental data which seem relevant to the hypothesis that the light period determines outward morphological expression through some control exercised by internal physiological conditions, will be discussed at this point. Morphological aspects of photoperiodism will be treated later in connection with the phenomena of growth and reproduction.

Several interesting experiments which throw light upon the nature of photoperiodic induction have been reported by Garner and Allard (147). Different portions of the main stem of *Cosmos sulphureus* (a short-day plant) were exposed to different daily periods of illumination and, in some instances, to continuous darkness. When the upper portion was exposed to long days while the lower region received only 10 hours of light daily, the former remained vegetative while the latter flowered promptly. Conversely, flowering in the upper portion was obtained by exposure to short days, while vegetative growth continued in the lower region exposed to long daily light conditions. When the upper portion of the plant was kept in continuous darkness for a period of 3-5 weeks, and the lower portion was forced into flowering by short days, flower buds were induced in the upper darkened portion. When the lower portion was prevented from flowering by long days and the upper part was kept in continuous darkness, the latter formed no flower buds. It was concluded that darkness in itself does not initiate flowering, but also does not inhibit the formation of flower buds in response to the action of an appropriate daily light period in another part of the plant. There remains, therefore, the distinct possibility that certain materials favorable

to flower formation may be formed in one part of the plant, and thence may be transported to another part where important processes are set into action leading toward initiation of flower primordia.

The localization of photoperiodic stimulation in several tuber-forming species has been studied by Rasumov (425, 427). Stimulation of the apical growing region by short-day exposures influenced the developmental trend of the whole plant. When the upper or lower parts of the same branch were subjected to a short day, the stimulus was delayed in the upward direction but transmitted readily downward. When the apex of *Ullucus tuberosus* was darkened, the axial buds were able to grow out into long branches. These outgrowths were capable of assuming a short-day habit by induction and their developmental trend was reversed when freed from the photoperiodic influence. Recalling the action of the plant growth-substance in inhibiting development of lateral buds in *Vicia faba* (536), these experimental results of Rasumov suggest that possibly some aspects of photoperiodism are concerned with the activity of hormones. Our knowledge is as yet too meagre to permit any definite conclusions regarding the nature of the formative materials and their manner of translocation.

In the course of experiments on "photoperiodic adaptation" in Russia, several workers observed that when plants were grown for more than a certain minimum number of days during the early part of their lifetime in a given daily light exposure, and later were transformed to a different daily light period, the effect of the first photoperiod was carried over and exerted an influence upon the subsequent development of the plants (112, 103). According to Dolgushin (103), Maximov called this phenomenon the "photoperiodic after-effect." It was postulated that the accumulation of substances in the plants retards or stimulates the transition into the reproductive stage. Rasumov (423) performed experiments with millet, a short-day plant, which was grown for different lengths of time during the early stages of its life in short or in long days and then placed for the remainder of the time in long or short days, respectively. It was concluded that preliminary exposure to a certain photoperiod exerted some influence which was later manifested in the development of the plants toward maturity. Recent workers have reported that only a short-day after-effect is

possible for short-day plants, and only a long day after-effect exists for long-day plants (74). The data of Rasumov and others have been examined critically by Purvis (416) from the viewpoint of the relative efficiency of long and short daily exposures in producing flower primordia. Miss Purvis' interpretation may be summarized as follows: In the short-day type of plant, such as millet, attainment of the condition termed "ripeness to flower" (*cf.* 241) may be reached under long or short-day conditions, but five times as rapidly under short as under long-day treatment; that is, one short day is equivalent to five long days in efficiency for inducing flower formation. Though short days hasten differentiation of flower primordia, nevertheless, subsequent stages of development leading over into actual flowering are independent of day length. In the case of a long-day type of cereal, like oats or barley, the development of the condition "ripeness to flower" is less dependent on day length than in a short-day plant. In barley, differentiation of flower primordia may be accomplished in about 10 days under either long or short days, but later stages of development into mature flowering condition are considerably hastened by long days.

Lubimenko and Szeglova (287) grew long-day plants, *Hordeum vulgare* and *Sinapis nigra*, and short-day plants, *Phaseolus vulgaris* and *Soja hispida*, under different day lengths in different phases of their life cycle. They found that induction of a long day retarded development of short-day plants when permitted to continue growth under short days, and accelerated long-day plants if they were later placed under short-day treatment. When seeds were germinated and grown in darkness for 5, 8 and 10 days and then placed in long-day conditions, growth was retarded in long-day plants and accelerated in short-day plants. The authors proposed to explain the photoperiodic induction by the photochemical formation and destruction of specific stuffs which influence directly the progress of development in the whole plant and its diverse organs.

Leading suggestions on the matter of special substance and photoperiodism have come from Lysenko (293), a strong advocate of "jarovization" (*cf.* 585). This investigator states that long-day plants should really be called plants requiring continuous illumination, and that they will endure alternation of light and darkness only in case the dark period is short. Requirement for alternation

of light and darkness is inherent only in short-day plants, but this characteristic may be overcome by suitable procedures before the seeds are planted. It is claimed that by preliminary treatment (jarovization) of the seeds of short-day plants under standard conditions of moisture, temperature, darkness and light (292, *cf.* 585), both increased vegetative growth and accelerated reproductive development may be achieved even under conditions of continuous light during the growth period. The requirement for darkness, without which short-day plants such as millet cannot pass into the reproductive stage, may thus be imparted to the plant during germination (*cf.* 24). McKinney and Sando (313) found that reproduction in winter wheat was greatly accelerated by subjecting slightly germinated seed to low temperature in darkness for 50 to 65 days before sowing. Change of a winter wheat to a spring wheat by preliminary seed treatment was reported as far back as 1858 in Ohio according to these authors (313). In a discussion of some of the more important relations of plants to light and temperature, Blackman (36) has pointed out that preliminary seed treatment accelerates the onset of the reproductive phase so that during its later growth the plant is largely independent of photoperiodic conditions. Even potato tubers may be "javorized" by exposure to daylight plus supplementary light during the night at a temperature of 15° to 20° C.

Recent investigations dealing with the effect of low temperature and light conditions on seed stalk development in vegetable crops are pertinent to the present problem. Thompson (538) has given a good discussion of the induction of seeding in celery by preliminary low temperature treatment. At high temperature Miller (323) was able to maintain active vegetative growth in cabbage over a period of several years, but the same plants were forced into flower by a few months' exposure to low temperature in a cool greenhouse. Though no definite relation between sugar and nitrogen composition and subsequent behavior could be shown, there appeared to be a positive relation between the accumulation of elaborated foods in the meristematic region and seed stalk formation. Platenius (396) investigated the metabolism in vegetative and prematurely seeding celery plants at different temperatures and found that the C/N ratio varied from .5 to 14.2 in the "seeders" and from 3.1 to 7.4 in the "non-seeders." In young plants the C/N

ratio was varied over a wide range without affecting the tendency to form seed stalks, and the consistently higher ratios of C/N obtained in the later stages of development were considered as the result and not the cause of the morphological changes. Chroboczek (79) found that beets grown in a cool glass house developed seed stalks under an 8-hour light day, while in the warm house even strong continuous illumination induced seeding in only a small percentage of the plants. A combination of low temperature (50°–60° F.) and long photoperiod (15 hours or more) is the most favorable for the production of large plants and high yield of seed. Beets grown in a cool greenhouse under continuous illumination produced seed stalks in 53 days from planting, but when kept at a temperature above 60° F., even under a photoperiod of 13 to 15 hours, the plants remained in a vegetative condition for 3½ years. Peto (388) found that high temperature favored vegetative growth while low temperature stimulated sexual reproduction in Swede turnip (*Brassica napus* var. *napobrassica*). The data obtained by Knott (243) for photoperiodic response and the facts gotten by Chroboczek (79) for morphogenetic effect of temperature, point to the meristematic tissues as being the seat of processes which determine the course of development in flowering plants. The recent attempts by Knott (245) to induce flowering in spinach by the application of localized light on the growing point, have given negative results due, no doubt, to the small area which was subjected to the light treatment.

#### REDUCTION/OXIDATION RATIO

The vigor and extent of vegetative growth taking place before the onset of reproduction in different species and individuals vary within wide limits, depending upon the various internal and external factors. As has been suggested upon several occasions, the relationship between income and outgo of oxidizable organic materials is a matter of considerable importance in the survival of plants under different conditions of light and temperature. Many evergreen plants appear able to assimilate carbon dioxide at a rate sufficiently rapid to maintain a favorable balance over the respiration of carbohydrates even at temperatures near freezing (585, 220). All other conditions being favorable, most plants seem capable of surviving under conditions of relatively low light inten-



sity, *i.e.*, in the range of from 1 to 5 per cent of full sunlight which approximates the light value where photosynthesis just balances respiration (the compensation point) (490, *cf.* 492). True shade plants are capable of existing for a comparatively long time under conditions unfavorable for photosynthesis. It is significant that shade-loving plants possess a relatively low respiration rate (291) in view of the fact (as Hendricks and Harvey (194) pointed out long since) that the light intensity required for the continued growth of a plant must be such that assimilation will at least over-balance loss by respiration.

Eaton (109) grew three series of each of several different species of plants in a 13-hour day and subjected them at night to temperatures of 90°, 65° and 50° F., respectively, to test the assimilation-respiration balance. Soy beans flowered earlier in high, and latest in the low temperatures, while cotton flowered earlier at the highest temperature but could make no growth at 50° F. In soy bean the greatest dry weight was formed in the cold night conditions, while the greatest final height occurred in the hothouse. Boysen-Jensen (50), from studies of the growth of light and shade plants, has shown that the quotient for maximum carbon assimilation/respiration yields a value of from 6 to 8 for sun plants and 10 to 12 for shade plants (*cf.* 486). Gabrielson (134) found the ratio for *Cucumis sativus* grown at 20° C. to be as high as 17.5, while the ratio may be still higher in other plants, *e.g.*, 25.0 in *Sinapis* (339). Lubimenko and Szeglova (286) found that the maximum dry matter per hour of light exposure was produced in a number of plants in the optimum daily periods as follows: *Gossypium*—8 hours, *Soja*—8 hours, *Sinapis*—14 hours, *Papaver*—16 hours, etc. In some plants, oxidation processes are greater in comparison with reduction processes than in other plants, and these two groups may be distinguished physiologically by the ratio of respiration/assimilation. The different daily rhythm of CO<sub>2</sub> exchange by different photoperiodic types of plants has been suggested as a causal factor in their response to relative length of day (440).

Great differences in respiratory rates as well as in temperature coefficients for oxidative processes are known to occur in different species (321). In some plants a carbohydrate deficit appears with a day length up to 10 hours, while in others a deficit is seen only with a day length up to 6 or even 4 hours. It is thought that these



differences are dependent upon the character of the enzymatic apparatus of the cells concerned in the processes of reduction and oxidation, (285) which control the photosynthesis/respiration ratio. Generally, increasing the light exposure to about 18 hours daily results in a corresponding increase in living substance (429). Too short periods do not permit sufficient photosynthesis of basic food, and continuous illumination appears to exert a strong inhibition upon growth through "the photochemical transformation of plastic substances" (285).

That light may have some important part in the liberation of energy by oxidative processes in living cells, has been suggested by various workers but little evidence has been offered to show direct causal relationship. Spoehr (513) noted that the respiration rate of germinating seeds was higher in sunlight than in darkness and he attributed the effect to the higher oxidative power of the atmosphere during light exposure. Parija and Saran (381) have reported recently that green and albino leaves of *Aralia*, starved for more than 40 hours in darkness and then exposed to blue-violet radiation, show increase in sugars and in rate of respiration. Red light had no effect. The explanation was offered that light increased respiration by hydrolysis of the carbohydrate reserve. Van der Pauw (375) also found an increase in respiratory rate of certain algae which were illuminated with intensities sufficiently high so as to inhibit photosynthesis. Guerrini (174) reported that red, and to a less extent the yellow, green and blue rays enhanced the rate of  $\text{CO}_2$  evolution of *Saccharomyces cerevisiae* in a glucose solution, but these results may have been due to the temperature rather than to photochemical action. It should be mentioned here that under certain experimental conditions light is known to have a chemical influence upon respiratory enzymes in plants. In darkness, indophenol can take up carbon monoxide and become inactive. Then upon exposure to light the CO compound is dissociated to liberate the active oxidase. Cytochrome, the respiratory pigment acting as a carrier or hydrogen acceptor, is oxidized by the indophenol oxidase in accordance with the reactions worked out by Warburg and others (cf. 233). The reality of this light effect has been shown by the experiments of Tang (531), who found that inhibition of oxygen consumption by germinating seeds of *Lupinus albus* exposed to CO could be abolished by light. Irradiation of

the yellow ferment of yeast has yielded crystalline flavin (569), and the interrelationship of this vitamin and the enzyme has been worked out by Theorell (535).

#### ENZYMES

The rôle of light in relation to enzyme activity has been studied directly in vitro and also in connection with the dormancy and germination of seeds, sprouting of vegetative storage organs, etc.

Demkovskii (97) investigated the enzymes present in "jarovized" seed and found that the increase in activity of amylase, catalase and the proteolytic group proceeded at different rates. What the relationship is between the results of seed treatment and photoperiodic phenomena is not definitely known. We can suppose that the effects of temperature and light may be occasioned through the production of catalysers in the nature of enzymes, hormones, etc. Knott (244) investigated the catalase in spinach before and after lengthening the photoperiod, and found a rapid response as exhibited by an increase in the enzyme activity following a change to long days. In another paper this same author (243) reported a decrease in catalase in the apical portion of the stem of spinach and *Cosmos*, as the plants changed to a reproductive type of growth. If elongation of the floral axis ceased and vegetative growth was resumed, then a higher catalase activity was restored. Burge (61) found that *Spirogyra* exposed to light exhibited a greater catalase content than when kept in darkness. The diastase of *Aspergillus niger* was found to retain its activity in darkness and in red and green light, but the enzyme was destroyed by white and blue light according to Funke (138). Pincussen (388) observed that the destruction of diastase by light in the presence of oxygen proceeded most rapidly at the optimal reaction of the medium. Hutchinson and Ashton (218) have reported various retarding and stimulating effects upon the activity of amylase when irradiated with specific wave lengths.

It has been held by some investigators that polarized light accelerates the hydrolysis of starch (20, 298, 483, 484) in distilled water and in the living plant. The validity of these reports has been criticized by those holding opposing views (229, 428). By careful experiments, Navez and Rubenstein (348, 349) have shown that polarized light and ordinary light of the same intensity and

wave length have the same accelerating effect on starch hydrolysis when compared with controls kept in darkness. Holman (205) has described the influence of high light intensity upon the disappearance of starch from leaves which may have been due to their destruction of chlorophyll and the hydrolysis of the starch, or both.

Recently, Semmens (484) has reported the results of experiments in which a very strong beam of polarized light was made to fall upon a starch-filled hyacinth leaf. Very rapid hydrolysis of starch to sugar occurred in the light under the polarizing Nicol prism, resulting in the bursting of the guard cells, while only the usual conditions prevailed in the rest of the leaf exposed to ordinary sunlight.

Recent successful attempts to overcome dormancy of seeds and tubers have shown that resumption of growth is accompanied by increased enzyme activity (99). It has been shown, too, that several special compounds which are effective in overcoming dormancy are also capable of promoting the action of specific enzymes (80). Other workers have found that the peroxidase activity is highest in such plants as millet and barley when grown in the photoperiod which favors vegetative growth (74). The story of light in relation to enzyme activity in plants can not be told satisfactorily without further critical experimentation under well controlled conditions. The recent work carried out by Gates (153) with ultra-violet effects upon crystalline pepsin may be cited as an example of a precise type of experiment which should point the way for future investigations of enzyme photochemistry.

#### VITAMINS

Production of vitamins in plants has received considerable attention in recent years since the rôle of vitamins has been appreciated more fully in animal nutrition (*cf.* 559). Gunderson and Skinner (175) grew a pure culture of the alga *Chlorococcum* in a nutrient dextrose solution in complete darkness and found that vitamin *A* (or its provitamin) was synthesized in large quantities. Vitamins *B* and *G* were found also, though no *C* could be detected. Recent investigations on the synthesis of vitamin *A* in higher plants indicate that though synthesized in darkness, its formation is usually accelerated by light (30, 87, 166, *cf.* 468). Smith and Morgan (505) claim that chlorophyll is not a necessary intermediary for

the formation of carotene and lycopene or any other precursor of vitamin *A*. Fruits which develop carotene and vitamin *A* in direct sunlight, may form them also in darkness. Carotene may be considered the precursor of vitamin *A*, much as ergosterol is the precursor of vitamin *D* (334, 335). Lojkin (279) found that ultra-violet irradiated lettuce, alfalfa, spinach and soy beans (but not cabbage) developed a slight vitamin *D* content, but this method of imparting the vitamin to animals was less efficient than the direct irradiation of the animal. Clover and alfalfa hay, when cured in the sun, lose their vitamin *A* but increase their vitamin *D* content (450, 507, 508, 518). Rygh (451) found an abundance of vitamin *A* in hay which had been dried quickly. It has been shown that light acts directly or indirectly on vitamin *C* formation in *Hordeum* seedlings, its accumulation in etiolated seedlings upon illumination being more rapid than the process of chlorophyll formation. Giroud and others (160) have observed that the ascorbic acid (vitamin *C*) content parallels the concentration of chlorophyll in various parts of a green plant. Heller (193) claimed that a greater increase in vitamins *A* and *C* took place in germinating cereals exposed to the shorter wave lengths present in sunlight. According to Jansen (223), vitamin *B<sub>2</sub>*, a flavine, is decomposed by light. An interesting relationship between vitamin and enzyme may be portrayed as follows: Vitamin *B<sub>2</sub>* + phosphoric acid + protein = yellow enzyme (cytochrome) of yeast (535).

In view of the known functions of ultra-violet radiation in the synthesis of vitamin *D* and in calcium fixation of animals, recent investigations of the chemo-synthetic activity of ultra-violet radiation in plants are of interest. Benedict (25) reported increased dry weight and increased per cent of calcium in tomato, corn, soy bean, cucumber and nasturtium grown with the ultra-violet region, 290–310 mμ, as compared with control plants receiving no wave lengths shorter than 310 mμ. Stewart and Arthur (524) also reported an increase in ash, and in calcium or phosphorus in plants exposed to brief daily periods of ultra-violet radiation. Cabbage, known to be lacking in antirachitic properties, did not respond to the treatment. The authors thought that the ultra-violet effective in fixation of the ash constituents exerted its influence indirectly by activation of the ergosterol present in the tissues of the plants. The chemical difficulties involved in experiments of this kind make

it difficult to interpret data on the basis of ultra-violet being the causal agent of the observed effects. It should be remembered that many kinds of plants have been grown to maturity in the complete absence of ultra-violet radiation without exhibiting any outstanding differences from plants receiving solar ultra-violet. Popp and Brown (402) have stated that out of 31 reports dealing with the effects of special ultra-violet transmitting glasses in greenhouses, unqualifiedly favorable results were given in only 8 cases and the data for these were not given or were of questionable value. Only slight differences have been found even in the most favorable reports, so that the use of special glasses for greenhouses is not to be recommended.

#### SEED GERMINATION

Seed germination in many species of plants is affected by light. About 1200 kinds of light-sensitive seeds have been reported and these include many of considerable economic importance (237, 357), such as species of *Poa*, lettuce, tobacco, etc. Some light-sensitive seeds continue their dormancy even after prolonged storage and refuse to grow unless exposed to light during the germination period; many others lose their need for light as a consequence of processes taking place in the seed during storage (300, 356); and still others, like the tomato, germinate better in darkness than in light (532). The question arises as to how photochemical processes may influence seed germination.

Honing (208) observed great variation in the need of light for germination in the different pure lines of *Nicotiana tabacum*. Results with reciprocal crosses between races of this species indicated that the need of light is a dominant characteristic, not of the seed coat, but of the embryo. Processes of after-ripening and the precise nature of the treatment during experimentation were found to be important in the determination of light sensitivity of *N. tabacum* and *N. rustica* varieties. The findings of Goodspeed (167), that some varieties germinated readily in darkness, may have been due to differences of genetic constitution or in the methods of experimentation so as to yield results at variance with those of Honing.

Schroppel (477) found that the respiration rate of germinating seeds of *Nicotiana tabacum* decreased in darkness. After a short

period of continuous illumination, their respiration rose rapidly and subsequently there followed a rise in the catalase and peroxidase activity. Since in light the acidity of fatty seeds of *Nicotiana* and *Verbascum* was found to be increased, Gardner (142) interpreted the phenomenon as due to splitting of fats to acids and glycerol by lipolytic enzymes activated in the light. Brief exposures to light (about 1 second at 200 meter candles) have been found effective in hastening germination of *Poa* sp. (300). However, germination of *Mimulus ringens* seeds exposed to weak light for long periods has been found to vary with the intensity, no germination taking place below 1.5 foot candles (217). The quality and quantity of the incident radiation which penetrates through the seed coat depend in part upon its absorption curve (18, 249). With equal energy values, the stimulatory effect of different wave lengths was found by Kommerell (249) to be proportional to the quanta of energy absorbed in the germinating seeds of *Lythrum salicaria* and *Nicotiana tabacum*. Recent studies of germination in lettuce have indicated that the blue region of the spectrum not only does not overcome dormancy but may actually induce it in normal seed (130). While short wave lengths inhibit germination, longer wave lengths in the orange-red region are effective in promoting germination. Shuck (493) claimed that non-dormancy may be retained in light-treated lettuce seed by drying the moist seeds in darkness.

Effects of ultra-violet radiation upon seed germination and early growth of seedlings have been investigated by many workers with diverse results and conclusions. Over a period of years, extensive experiments have been performed by Popp and Brown (*cf.* 402) using turnip, radish, cucumber, pigweed and curled dock. Different bands of ultra-violet were employed for irradiating the germinating seeds and the effects were recorded by the general appearance of the cultures, hypocotyl lengths, leaf measurements and dry weights. No significant stimulation was ever obtained when adequate controls were used. In a critical review of the literature on this subject, these authors (402) state that the only fact clearly demonstrated by experiments thus far carried out on the effect of ultra-violet radiation on seed germination and seedling growth is the injurious effect of short-wave radiation below 290 m $\mu$ . Evidence from more carefully controlled experiments indicates little

or no effect of the longer wave lengths of ultra-violet, *i.e.*, those from 290 to 400  $m\mu$  which are found in sunlight.

Applicability of the Bunsen-Roscoe rule for promotion of germination in Arlington Fancy lettuce seed has been tested by Flint (130). Over a relatively low intensity range of Mazda light the product rule seemed to hold true, but over the range 2 to 2048 f. c. at standard exposures of 1 second, the per cent germination increased from 0 to 86. In a more recent paper, Flint and McAlister

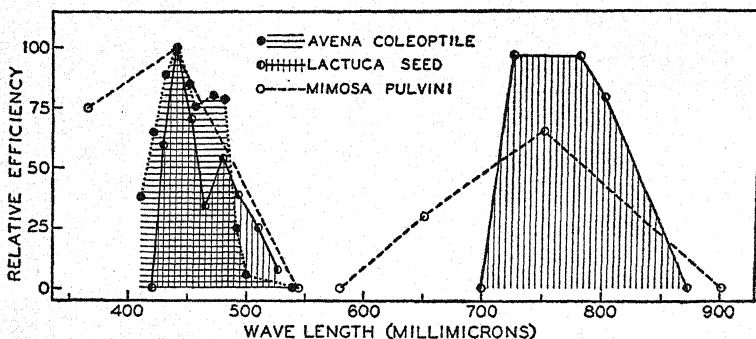


FIGURE c. The relative efficiency of different regions of the spectrum in bringing about phototropism, seed dormancy and photonasty in plants. Bending of the oat coleoptile toward light occurs chiefly in response to the shorter visible rays, as indicated by the dotted line and horizontal hatching. Germination of light-sensitive lettuce seed is inhibited by a band of radiation in the red and another in the blue region, as shown by the solid line and vertical hatching. The relative effectiveness of the short wave lengths of light in both of these phenomena is represented by bimodal curves with maxima in the regions 440 and 480  $m\mu$ . The induction of seed dormancy by red light is not paralleled by phototropic response to the same rays. However, the activation of *Mimosa* pulvini by blue-violet and also by red wave lengths shows some similarity to the effect of light upon lettuce seed. Further study might show a relationship between the processes involved in phototropism, photonasty and seed dormancy. Data for the *Avena* coleoptile have been taken from Johnston (228), for *Lactuca* seed from Flint and McAlister (131), and for *Mimosa* pulvini from Burkholder and Pratt (62)).

(131) have demonstrated that a band of radiation in the region of 760  $m\mu$  and another in the region 420 to 520  $m\mu$  inhibits germination of this same variety of lettuce seed. On the other hand, the yellow-orange wave lengths promote germination. The relative effectiveness of narrow bands of shorter wave lengths of visible light was found to be similar to the spectral sensitivity curve for phototropism in etiolated coleoptiles of *Avena* (228). It is significant that the spectrum curves for induced dormancy in lettuce



seed and phototropism in *Avena* should show maxima at about the wave lengths of 440 and 480 m $\mu$  which are known to closely approximate the absorption maxima of carotenes (cf. 322).

The long red wave lengths which inhibit germination are effective in provoking response in *Mimosa* (64). The action of light in phototropic phenomena is known to be concerned with the function of certain specific substances, auxins (cf. 247), and, moreover, nastic movements of *Mimosa* can be influenced also by application of hetero-auxin (247) to the pulvini (64). Whether auxins have any rôle in connection with the phenomena of dormancy and germination of seeds remains to be proven.

The necessity of water and available oxygen during exposure to light for the accomplishment of the light effect has been emphasized (39, 477, 586). Working with selected kinds of plants, Böhmer (39) found that germination of light-inhibited seeds was favored by a higher partial pressure of oxygen than that of the normal atmosphere, but that germination of light-favored seeds was inhibited by higher oxygen, while light-indifferent seeds remained unaffected by considerable variations in the oxygen partial pressure.

Light sensitive *Lythrum salicaria* seeds, which had been kept in an atmosphere of nitrogen or of hydrogen, showed much better germination subsequently than did seeds which had been kept in an atmosphere containing oxygen (541). Several investigators (cf. 39, 493) have obtained evidence suggesting the existence of a photosensitive substance in light sensitive seeds, but as yet no specific chemical substance has been identified in this connection.

#### GROWTH-SUBSTANCES

Experiments dealing with growth behavior of plants in relation to light have, in some instances, yielded results not to be explained on the basis of variation in supplies of fundamental constituents, such as minerals, water and carbohydrates. Searches for satisfactory explanations of certain puzzling phenomena have led to the discovery of many special substances including enzymes, vitamins, hormones, etc., which play specific rôles in physiological processes. Some effects of light which appear to be exerted through formation and activity of special growth-substances have been worked out in a rational manner in the last several years. Many early investigations, including those of Darwin, Sachs, Boy-



sen-Jensen, Paal, Söding, and others (*cf.* 52), gradually built up a fund of information which strongly suggested the existence of specific growth-promoting substances, but only in recent years has definite progress been made in the direction of their qualitative and quantitative determination. Went's (577) paper on the quantitative determination of growth-substance gave a new impetus to investigations which have yielded remarkable discoveries. At Utrecht, Kögl and his associates (247) have discovered and prepared in pure form several substances which promote plant growth, it is believed, mainly through increase in cell size. These substances have been called *auxins*. The relation of light to the rôle of growth-substance action in plants is as yet poorly understood. Went (577) found a decrease of 18 per cent in the amount of growth-substance given off by the coleoptile of *Avena* when illuminated with 1000 m.k.s. as compared with plants kept in darkness. Küstner (261) observed that the activity of growth-substance prepared from urine was increased by red light, and decreased by shorter wave lengths. Navez (347) was able to demonstrate an increased amount of growth-hormone in apical portions of *Lupinus albus* seedlings subjected to weak Mazda light. Etiolated *Raphanus* seedlings lose the ability to form growth-substance, while plants kept in the greenhouse retain their synthetic power for for a long time, according to Van Overbeek (374). Avery (17) found that a growth-hormone was produced in young growing leaves of tobacco in the light, but in darkness it disappeared after several days. The data of Chesley (75), who reported that wheat seedlings sprouted in the light were less sensitive to X-radiation (which destroys auxin (500)) than those germinated in darkness, may be interpreted on the supposition that a greater concentration of auxin was present in the illuminated plants, and hence greater doses of X-rays were required to check their growth. Went (581) has stated that "only in seedlings is growth-substance formed in the dark, apparently from reserves in the seed." That light may indirectly have some relation to the manufacture of substances which promote root formation may be inferred from experiments of Went (578) with *Acalypha* cuttings, where roots were formed in greater numbers when expanded leaves or buds were present on the shoots. In a later paper, Went (580) states that "in plants with leaves in the light, root formation goes on during weeks and

no constant level of root formation is reached, indicating that in leaves we have to do with new formation of rhizocaline," a root-forming substance. Red and orange wave lengths appear to be especially effective in the production of rhizocaline, though light seems to inhibit its action (582). Laibach (262), Müller (339) and others have shown that roots are formed abundantly on cuttings to which growth-substance has been applied. Presumably, light is essential for synthesis of growth-substance in plants, and the nature of photochemical reactions involved await thorough investigation.

#### ELECTRICAL POTENTIAL

The fundamental nature of the polar axis in plants has been one of the great morphogenetic problems since the time of Sachs (453) who postulated the presence of shoot- and root-forming substances to account for axial differentiation. Vöchting (560, 561) and others have demonstrated that not only gross structures but also individual cells of the plant body possess definite polarity. Experimental results of many investigators in recent years have given good reasons for believing that morphological polarity is related to electrical polarity.

Since electrical potentials in plants were described at length by Bose in 1907 (46), a vast amount of experimental data has accumulated on the subject (*cf.* 29, 274, 372, 522, 445). The power of light in the production of electric currents was very well demonstrated in one of the experiments described by Bose (47). Two halves of a banana leaf, severed along the midrib, were immersed in a dilute salt solution and wire leads were connected to form an external circuit through an electrometer. When one leaf portion was illuminated and the other kept in darkness, the system behaved as a photoelectrical cell and a flow of current was registered on the sensitive indicator. Waller (565) experimented with photoelectric effects in green, albino and etiolated tissues, and came to the conclusion that chlorophyll was the active agent in the responses observed. In a later paper by the same author (566), the bioelectric current was attributed to lack of equilibrium between oxidative and reductive processes. Most leaves showed an initial negative phase upon illumination but etiolated leaves and others kept in darkness for a number of hours gave an initial positive phase.

Sheard (489) has reported induced potentials which attained a maximum of the order of .1 volt in the leaves of sunflower and poinsettia exposed suddenly to ultra-violet or infra-red radiation. Glass (162) used excised green leaves of *Elodea* and measured the potential produced by an intense spot of light applied locally to different regions along the midrib. When the apex of the leaf was illuminated, a potential difference of about 100 millivolts was found between the apex and base, the former being positive in the external circuit. When the spot of light was applied to the base only, this region became positive (external circuit) to the darkened apex. Transmission of the effects of light to non-illuminated regions in the plant was obvious from the experimental results. The author was of the opinion that the effect of light upon E. M. F. in the leaf was not a direct photoelectric effect but, through action of light on the chloroplasts, changes of state were set up which gave rise to the electric phenomena. Experiments of Brauner (54) with *Hordeum* have shown that unilateral light sets up a negative potential on the lighted side. This discovery has considerable significance in the theory of tropisms which are now explainable on an electrical basis. Some of the ways in which electrical polarity may give rise to profound morphological effects in the plant will be referred to under the discussion of morphological differentiation.

That light may have direct action upon physical properties of the minute structure of the cell has been suggested by several investigators. Hercik (195, 196) interpreted the rise in surface tension of cell sap in etiolated seedlings after illumination as a photoelectric phenomenon similar to the Hallwach's photoelectric effect (*cf.* 592) where negatively charged bodies lose their charges upon illumination. When the negative particles of sap lost their charge in light and surface tension was raised, Hercik called it a positive photocapillary reaction.

Overbeek (374) has put forth a theory to explain direct inhibition of light upon cell-wall-stretching on the basis of photoelectrically altered charges on the intermicellar substance which is believed to be charged oppositely to that of the cellulose layers of the wall so as to hold the pattern in a more or less rigid position. According to Overbeek's theory, the light quanta which are absorbed by cell walls would tend to increase the potential difference between the cellulose and intermicellar substance, and thus render the wall

less stretchable and less susceptible to the action of growth-substance. It may be of interest in passing to mention that Chouchak (77) found that in the light, leaves bearing a positive charge absorbed more  $\text{CO}_2$  than those having a negative charge, thus influencing the process of photosynthesis.

To account for polar distribution of growth-substance, Went (579) proposed the theory of electrical potential in plants, according to which the charged particles or ions are attracted in the direction of unlike charge. Recently, distribution of the growth-hormone in plants has been demonstrated experimentally on the basis of electrical potential. Ramshorn (419) and Koch (246) found that negatively charged ions of growth-substance migrated toward the positively charged region in the organs of the plant (as it did also in agar) and there exercised its rôle in causing tropistic growth responses. Since light is known to be active in the formation of potential gradients in plant tissues, it is obvious that the light factor must have a significant effect upon growth by exercising an indirect influence upon distribution of specific growth-substances.

In view of modern developments in this field of research, it is apparent that light has profound influence upon polarized and general growth, and upon processes concerned in differentiation of the plant body; but the mechanism is by no means completely understood. Growth and developmental characteristics which appear as responses to variations in the light environment suggest specialized types of internal chemical reactions and physical conditions, but it is difficult to discover the latter and show the complete history of the case from environmental stimulus to morphological response.

A profound morphogenetic influence of light upon the polar growth gradient in higher plants has been suggested by recent work concerning growth and inhibition of lateral buds by auxin (536). Also Kahane (230) has shown that either in the presence or in the absence of  $\text{CO}_2$ , light could condition normal development of axillary buds of true leaves and repress the cotyledonary buds in pea seedlings, while in darkness, vigorous growth occurred from the axils of the cotyledons. Garner and Allard (146) pointed out that in a day length below optimum, apical dominance may be diminished so that the leaf rosette or branching habit develops in place of the erect form. In *Cosmos* and *Poinsettia*, grown under

long days, there was a tendency for foods to go toward the upper part of the plants. Under favorable light conditions, carbohydrates stored earlier in tubers and thickened roots were translocated upward for growth in stature of the shoot. Experiments with *Ullucus tuberosus* and *Oxalis tuberosa* (425) have indicated the possibility for certain effects of localized photoperiodic stimulation to be readily transmitted morphologically downward but not upward. Investigations of Garner and Allard (146, 147) have given indication of transmitted photoperiodic influence only under certain specific conditions. Proof of the formative effects exercised through electrical potential has recently been given by important experiments of Schechter (467), working with the red alga *Griffithsia*. This investigator was able to bring about regeneration of rhizoids on the induced positive end, and vegetative shoots on the induced negative end of isolated portions of the alga grown in sea water in an electro-culture chamber. From the experimental results, it may be inferred that formative substances may be differentially distributed under conditions accompanying normal polarity in plants, and in this manner differential growth patterns may be initiated and maintained. Recent discoveries concerning the hormonal nature of the cambial stimulus (510, 511) and the downward movement of cambial activation in woody plants (412, 537) suggest that light, through its influence upon growth-substance formation, and electric polarity, through its action in causing differential distribution of these ionized substances, may be of much greater importance for morphogenesis than hitherto realized.

*Bibliography will appear with the second part of Dr. Burkholder's article in the March issue.*

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# THE BOTANICAL REVIEW

VOL. II

FEBRUARY, 1936

No. 2

## THE RELATIONSHIPS OF THE HEPATICAE

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The Hepaticae, as usually understood, comprise four orders: Anthocerotales, Sphaerocarpales, Merchantiales and Jungermanniales.

The Anthocerotales, however, while probably related distantly to the other Hepaticae, differ so greatly in the structure of both gametophyte and sporophyte that their separation, as a class, Anthocerotes, seems amply justified.

The single family, Anthocerotaceae, is a very natural one, but its relationships with the other Archegoniates are by no means clear and its relations to the other Bryophytes are very obscure. There are four genera, *Anthoceros*, *Megaceros*, *Dendroceros* and *Nothothylas*. The largest genus, *Anthoceros*, has been separated into two by Stephani. There are over 200 described species in the family, wide spread in the warmer parts of the world.

The gametophyte is a prostrate thallus, composed of nearly uniform cells, in most cases containing a single chromatophore, which closely resembles that of the Ulothricales and often contains a conspicuous pyrenoid. This alga-like chromatophore is not known elsewhere among the Archegoniates.

While the gametophyte of the Anthocerotaceae most nearly resembles the green algae from which it is believed the higher plants have been derived, the sporophyte is much better developed than in any of the Hepaticae, and is in some respects more like that of the mosses, or even the most primitive of the pteridophytes. Thus, the Anthocerotes may be said to form a synthetic, and presumably, very ancient group of plants with relationships on the one hand with the bryophytes and pteridophytes and, on the other, with the green surface and vertical rows

It is not impossible that from the same stock as the Anthocerotes, the Hepaticae have been derived. Sometimes, as in *Megaceros*, the single chromatophore is replaced by several, and may be compared with those of some Hepaticae, like *Cyathodium*, for example. In Anthocerotes, unlike the other liverworts, the sporophyte is not only a spore-producing structure, but the sporogenous tissue, especially in the higher forms, like *Anthoceros*, is subordinated to the vegetative tissues. The sporophyte may live for months after the first spores are discharged, and the presence of a zone of meristematic tissue at the base enables it to increase in size, developing both new sporogenous and vegetative tissues. Abundant chlorophyllous tissue is present, and in some cases an efficient water-conducting tissue—thus approaching the independent condition found in the pteridophytes (1, 7, 12). A comparison has also been made between the sporophyte of *Notothylas*, the least specialized of the Anthocerotaceae, and that of *Cyathodium* or *Sphaerocarpus*, two of the simplest Hepaticae. It has been held that the Anthocerotes were derived from such simple Hepaticae through forms like *Notothylas*. It may be said, however, that admitting a real relationship between the Anthocerotes and Hepaticae, it seems more likely that the Anthocerotes are the older types and the Hepaticae the derivative ones. The sporophyte of the first Anthocerotes must have been much simpler than that of any of the existing forms—perhaps comparable to that of such liverworts as *Riccia* or *Sphaerocarpus*.

Excluding the Anthocerotes, the remaining liverworts show sufficient evidences of relationships among themselves to warrant, for the present at least, their inclusion in a single class, Hepaticae.

While there is good reason to assume that some of the Hepaticae are very old types, very little is known at present of their geological history, and the few known palaeozoic fossils are referable to existing types (32, 33). While certain groups are sufficiently well defined, e.g., Sphaerocarpaceae, Ricciaceae, the relationships of these groups to each other, and the interrelationships of the members of the larger orders, are not always so evident, and it can hardly be said that an entirely satisfactory classification of the Hepaticae has been established. Three orders, Sphaerocarpaceae, Marchantiales and Jungermanniales, may be recognized (14) and possibly a fourth, Calobryales, may be added (11). *Calobryum* the type



of the Calobryaceae, has been referred to the Jungermanniales, but differs essentially from the latter in the character of both the sexual organs and the sporophyte.

The simplest gametophytes of the Hepaticae closely resemble that of *Anthoceros*, a prostrate thallus composed of similar cells throughout, e.g., *Aneura*, *Pellia*. From this undifferentiated thallus there have been developed several specialized types, evidently arising independently in a number of divergent lines of evolution. In the Jungermanniales one type, e.g., *Metzgeria*, *Pallavicinia*, there is a definite midrib which may have a central strand of elongated conducting cells, sometimes with thickened cell walls, recalling the tracheary tissue of vascular plants. The lateral wings of the thallus consist of a single layer of chlorophyllous cells. Sometimes, e.g., in species of *Pallavicinia* and *Hymenophyton* (*Umbra culum*), there is a differentiation into a prostrate cylindrical rhizome-like portion from which upright branches arise, undergoing repeated dichotomy so that these upright shoots resemble the palmate fronds of a filmy fern. In some species of *Aneura* there is a central axis with numerous lateral branches—thus suggesting a pinnate fern-frond.

In another category are the leaf-like lobes found in a number of liverworts belonging to quite unrelated families. These lobes may be only slightly developed, e.g., in *Blasia* and some species of *Pallavicinia*, or they may develop into definite leaves, as in *Treubia* and *Noteroclada* whose leaves bear a definite relation to the apical cell of the shoot, as they do in the leaves of the acrogynous Hepaticae, the "foliose" or leafy liverworts. In most of the latter, the leaves form three definite series, corresponding to the segments cut off from the tetrahedral apical cell. The leaves in these Acrogynae are often complicated in structure.

The evolution of the gametophyte has been quite different in the Marchantiales. The strictly thallose form has been retained, but there has been a very marked differentiation of the tissues, culminating in such highly specialized types as the Marchantiaceae, e.g., *Marchantia*, *Fegatella*. The most primitive condition is found in some species of *Riccia*. In *R. glauca* the dorsal tissue of the thallus develops a system of narrow air-chambers opening at the surface and surrounded by the chlorophyllous cells which are in vertical rows. The ventral region is composed of compact tissue



which passes gradually into the green dorsal tissue. In the more specialized Marchantiaceae there is a definite epidermis with characteristic pores communicating with a system of air-chambers to which the green tissue is confined. In *Marchantia* and *Fegatella* these air-chambers form a single layer, sharply separated from the solid ventral tissue. Each air-chamber has a single large pore.

In some Hepaticae there is a preliminary structure, the "protonema," developed from the germinating spore, and the definitive gametophyte arises as a bud or branch from the protonema.

The sexual organs, archegonia and antheridia, may develop directly from the thallus, or there may be special receptacles developed upon which these are borne in the higher Marchantiales, like *Marchantia* and *Dumortiera*. The Hepaticae present a strong contrast to the Anthocerotales, in the variety shown by the gametophyte and the relatively highly specialized structures shown by some of them. On the other hand, the sporophyte is much less developed than that of the Anthocerotaceae and remains to a great extent parasitic upon the gametophyte. In its simplest form, e.g., *Riccia*, it is merely a capsule with a single layer of wall cells enclosing a mass of spores. In the most highly developed forms, it shows an elongated stalk (seta) bearing the globular or cylindrical capsule. In addition to the spores there are the sterile cells, elaters. Practically no chlorophyll is present and the developing sporophyte is dependent upon the gametophyte for its growth. With discharge of the spores the tissues collapse and wither away. It thus offers a marked contrast to the long-lived and nearly self-supporting sporophyte of *Anthoceros*.

#### SPHAEROCARPALES

This small order, containing three genera and about 20 species is, on the whole, the simplest of the Hepaticae. The type genus *Sphaerocarpus* has several species on the Pacific Coast and in the southeastern states. *Sphaerocarpus* is dioecious, the males being much smaller than the females. The gametophyte is a simple thallus, composed of uniform cells, the central portion forming an indefinite broad midrib which merges gradually into the lateral wings composed of a single layer of cells. The sexual organs, each enclosed in a conspicuous involucre, cover the dorsal surface of the thallus.

The sporophyte is intermediate in structure between that of *Riccia* and that of the typical Marchantiales. Of the two cells resulting from the first division of the zygote, the upper (epibasal) cell gives rise to the globular capsule, the lower (hypobasal) to the haustorium (foot). Unlike *Riccia*, where all of the inner cells of the capsule produce spores, in *Sphaerocarpus* some of the sporogenous cells remain undivided, but do not develop into the elaters of the typical liverwort sporogonium.

The second genus of the Sphaerocarpaceae consists of *Geothallus tuberosus* (5), a monotypic genus from southern California, differing from *Sphaerocarpus* in its much larger size and the development of definite leaves, much like those of *Fossombronina*, one of the Jungermanniales, with which the Sphaerocarpaceae were formerly associated. The third genus, *Riella*, while agreeing with *Sphaerocarpus* in the structure of the sexual organs and sporophyte, differs greatly in its habit, being a submersed aquatic. Most of the species of *Riella* occur in the regions adjacent to the Mediterranean, but one occurs in the United States, and others in the Canary Islands, and South Africa.

While the Sphaerocarpaceae were formerly associated with the Jungermanniales, they were later separated (14) as an order coordinate with the Marchantiales and Jungermanniales, and to some extent intermediate between them.

#### MARCHANTIALES

The Marchantiales, comprising about 400 species, form a very clearly defined order. Most of the genera and several species are cosmopolitan.

The gametophyte is always a prostrate thallus, commonly branching dichotomously, and there is no development of leaf-like photosynthetic organs so characteristic of the more specialized Jungermanniales. The thallus, however, in most of them, shows a remarkable degree of differentiation of the tissues. Usually the massive thallus has the ventral region composed of compact tissue with little or no chlorophyll present, while the green tissue is confined to the dorsal region. In the less specialized genera, like *Riccia*, there is a transition from the green dorsal tissue to the colorless ventral region. In highly specialized genera, as *Marchantia* and *Fegatella*, the chlorophyllous tissue is sharply segre-

gated and there is a single tier of dorsal air-chambers, or lacunae, which open at the surface through characteristic pores (stomata) in the epidermis. The green tissue occupies the floor of the air-chambers from which short rows of green cells extend into the air-chamber and thus form a very efficient photosynthetic apparatus.

Another and less specialized type is found in some other genera, e.g., *Fimbriaria*, where the lacunae are large and irregular in form and the lacunar region is not clearly delimited from the compact ventral tissues.

The genera *Monoselenium*, *Dumortiera* and *Monoclea* differ from the typical Marchantiales in having the thallus, like that of *Anthoceros* or *Aneura*, composed of uniform green tissue with no lacunae. In *Monoclea* and *Monoselenium* there is no trace of the air-chambers but in some forms of *Dumortiera* there are evident remains of dorsal air-chambers which become almost completely obliterated. It is generally assumed that these genera are not primitive but have been derived from forms which possessed such air-chambers.

The Marchantiales are characterized by the presence of membranaceous scales developed on the ventral surface of the thallus. These scales are usually in two rows. Some of the rhizoids have thickened cell walls with conspicuous spike-like protuberances on the inner surface.

#### THE SEX ORGANS

The archegonia and antheridia are much alike in all the Marchantiales. The archegonium has six peripheral rows of neck cells, and the antheridium has a short pedicel. In *Riccia*, the archegonia and antheridia are more or less mixed, and are formed directly from the superficial cells of the thallus. In the most specialized genera, like *Marchantia*, the plants are dioecious and the reproductive organs are formed in greatly modified receptacles, borne on slender pedicels. There are various intermediate conditions between *Riccia* and the higher Marchantiaceae. Among these, the small family Corsiniaceae with the genera *Corsinia* and *Boschia*, may be said to connect the Ricciaceae and Marchantiaceae. In the latter, the female receptacle, or carpocephalum, is formed at the apex of the thallus by a rapid dichotomy of the apex and is, there-

T1

branch system, each apex developing one or more fore, a comp

archegonia. In Marchantiales does the sporophyte attain a degree

In none corresponding to that of the gametophytic structure of of speciali of them the wall of the capsule consists of but a few layers. In few cells, except that in some cases there is a more or less single layer, by which the capsule opens; but as a rule the capsule thickens irregularly. Except in the Ricciaceae and Corsiniaceae, wall is formed and there is a short seta and foot. The three elaters Ricciaceae, Corsiniaceae and Marchantiaceae form an families series, showing the progressive evolution of the sporogone-interes (26).

Among these three families there are two others whose relation

Band to each other are not so clear. They probably represent two independent lines of development originating near the base of the Marchantiales. It has been suggested that they are connected with the Ricciaceae through the Corsiniaceae, but it is not impossible that they may have been derived from forms more nearly related to the Sphaerocarpaceae. The Targioniaceae have two genera, *Targionia* and *Cyathodium*. The only American representative is *Targionia hypophylla*, common on the Pacific Coast but not found in eastern North America. This species is widely distributed, occurring also in Europe, South Africa and Australia. The structure of the thallus is much like that of the Marchantiaceae but no carpocephalum is developed.

*Cyathodium* has about a half-dozen species in moist tropical regions, growing in shady locations, like the openings of caves. The thallus is very delicate in texture and the chloroplasts are large and few in number.

#### MONOCLEACEAE (4, 14, 20, 29)

This small family contains but two species—*Monoclea Forsteri* of New Zealand and Patagonia, and *M. Gottschei* from tropical America. There is much difference of opinion as to the systematic position of *Monoclea* but the weight of evidence indicates that it should be included in the Marchantiales.

In general appearance the gametophyte resembles a large *Anethoceros* or *Aneura*, the thallus being composed of uniform soft tissue with no trace of the characteristic air-chambers of typical German-

Marchantiales. Moreover, the sporophyte has a type of Mar-  
like that of most Jungermanniales. However, develop-  
sexual organs and the sporophyte conform to the relationship  
Marchantiales rather than to that of Jungermanniales (20). The lack  
of air-chambers is found also in *Dumortiera*, which may be asso-  
with the higher Marchantiaceae is beyond question. Monocleaceae  
*Forsteri* often grows actually partially submersed and, near the  
assumed that the absence of air-chambers may perhaps be asso-  
ciated with this hygrophilous habit. Possibly the Monocleaceae  
should be placed at the beginning of the Marchantiales  
point where the Marchantiales and Jungermanniales diverge from  
some common stock.

#### JUNGERMANNIALES

A recent enumeration of the Hepaticae shows that of a total of  
8538 species, 7803 belong to the Jungermanniales. The order  
seems to be a natural one but the further classification is in a very  
unsatisfactory condition. There is much difference of opinion as  
to the limits of the families and the genera belonging to them.  
The classification has been based largely upon external characters  
and only a relatively small number of species has been studied  
critically as to their life-history and, especially, the development of  
the sporophyte. Until much more has been done in this direction,  
any proposed classification must remain to a great extent merely  
tentative.

Of the classifications that have been proposed, perhaps that of  
Cavers (14) is the most satisfactory. It follows, in the main, that  
of Schiffner (28) but differs in some important respects. To  
quote Cavers: "The Jungermanniales form a single phylum, the  
boundaries between the systematic families in most cases badly  
defined, and probably in no other group of plants do we find such  
striking and abundant examples of parallelism or homoplasy."

The gametophyte may be, on the one hand, a simple prostrate  
thallus, while with most specialized types there is a definite axis  
bearing leaves which have a direct relation to the segments of the  
apical cell. Between these extremes are many intermediate condi-  
tions, and it is evident that very similar structures have arisen in  
several independent lines of development. The tissues in the great  
majority of the Jungermanniales are very uniform and there is

nothing comparable to the highly specialized tissues of the gametophyte in the higher Marchantiales.

The sporophyte has a definite capsule and seta—the latter often much elongated—and sometimes a conspicuous foot. The wall of the capsule has two or more layers—thus differing from the unistratose wall in the Marchantiales. The spore-mother-cells become deeply four-lobed before the first nuclear division. Elaters are always present.

The Jungermanniales have been divided into two series—Anacrogynae and Acrogynae—based primarily upon the position of the archegonium. This division is somewhat artificial as there are some intermediate forms.

In the Anacrogynae the archegonia are borne upon the dorsal surface of the gametophyte and further growth of the shoot is not affected. In the Acrogynae the apical cell of the shoot is finally transformed into an archegonium, and further growth is thus prevented. All of the Acrogynae develop leaves and include the great majority of the liverworts.

#### ANACROGYNAE

Cavers recognizes four families of Anacrogynae: Codoniaceae, Aneuraceae, Blyttiaceae and Calobryaceae. Goebel (16) unites the second and third of these and part of the first into a single family, Metzgeriaceae, and proposes three additional families—Pelliaceae, Fossombroniaceae and Treubiaceae. This illustrates the very unstable condition of the present classification of these Hepaticae.

About 600 species of the Anacrogynae have been described. As already indicated, the gametophyte may be a quite undifferentiated thallus, e.g., *Pellia*, *Aneura pinguis*, or there may be developed a definite midrib with conducting tissue, e.g., *Pallavicinia*, and various types of leaf-like organs. The development of frond-like branch systems, shown in *Umbraculum* and *Mittenia*, is an interesting case of homoplasy so characteristic of the Anacrogynae.

#### CALOBRYACEAE (11, 16)

This small family, with only about half a dozen known species, is usually included in the Jungermanniales, but it differs so markedly from any of the other families that it would seem better to establish an order, Calobryales, coordinate with the Jungerman-

niales, Marchantiales and Sphaerocarpaceae. The best known form, *Calobryum Blumei*, has a prostrate rhizome-like stem from which are developed upright shoots bearing three series of conspicuous leaves. The habit is much like that of some acrogynous liverworts, and the apical growth is much the same. However, the structure of the sexual organs and sporophyte differ greatly from those of any of the other Hepaticae and the family seems to be quite unrelated to any other family of liverworts.

#### ACROGYNAE

The Sphaerocarpaceae, Marchantiales and anacrogynous Jungermanniales are, presumably, old groups of which relatively few forms have survived. Some of the latter, as we have seen, develop leaf-like organs which may closely resemble those of the Acrogynae, and it seems probable that the latter have been derived from some types related to these foliose Anacrogynae. It is highly probable that the existing Acrogynae represent several independent phyla derived from different anacrogynous ancestors.

While there are more than 7000 species of Acrogynae, some seven times as many as all the other Hepaticae, their structure is much more uniform than that of either the Marchantiales or the Anacrogynae. The gametophyte is dorsiventral and has a definite central axis bearing usually three rows of leaves corresponding to the three series of segments formed from the tetrahedral apical cell. Sometimes the ventral row of leaves ("amphigastria") is absent.

The Acrogynae are decidedly the predominant liverwort type, occurring in all parts of the world but reaching their maximum development in tropical mountain forests and moist lowland forests of the southern hemisphere where they form a conspicuous feature of the flora. They may be strictly terrestrial in habit, growing also on rocks or fallen logs, or they may be epiphytes. The epiphytic habit is especially marked in tropical rain forests where they occur not only on the trunks and branches of trees, but many small species grow on the surface of fern fronds and other broad leaves. These "epiphyllous" species are especially abundant in the rain forests.

The epiphytic habit is probably a secondary development and it may be assumed that the terrestrial condition, like that of most



Anacrogynae and Acrogynae and systematic position of *Podomitrium*. Am. Jour. Bot. 3: 261-273. 1916.  
 acrogynous genera, I. Archegonium and sporophyte of *Treubia insignis* in some East Indian Hepaticae, *Calobryum* quite possible that fr. Ann. Botany 34: 1-12. 1920.  
 the Acrogynae may have a remarkable development of the sporophyte in *Anthoceros*. Aust. Ann. Botany 38: 473-483. 1924.  
 size, and the leaves small. GAILLOIT FLORENCE. A morphological study of some development of special water sacs in *Frullania*. Stanford Univ. Press, 1914.  
 very little specialization and there are no definite types. Trans. W. A. K. or variations within it, indicates a highly specialized and presumably relatively modern group, compared with the anacrogynous types.

Just as among the Anacrogynae there occur genera like *Treubia* and *Androcryphia* which suggest the Acrogynae, so among the latter there are genera which, while truly acrogynous, show a marked tendency to develop a thallose condition such as is typical of the Anacrogynae. In these forms the gametophyte at first develops a sort of protonema from which the fertile leafy shoot arises secondarily as a bud. This protonema is sometimes a branched alga-like filament like the protonema of a moss. In other cases it is a flat thallus resembling the simpler Anacrogynae.

The sexual organs do not differ essentially from those of the Anacrogynae. Development of the complete sporophyte is known in only a small number of species, but two markedly different types have been described, and on this basis the Acrogynae have been divided into two "tribes," Jubuleae and Jüngermanniae. The differences between the sporophytes in these two groups are such as to suggest that they represent two independent phyla. In the first occur the very large genera *Lejeunea* and *Frullania*, in the other, all the other genera that have, as yet, been investigated (30).

#### INTERRELATIONSHIPS OF THE HEPATICAE

The Hepaticae apparently represents a natural group whose most specialized members are the Marchantiaceae and the acrogynous Jüngermanniales—the latter being the most recent. Assuming that the Hepaticae are all more or less related, the simplest, and presumably most primitive of the existing forms, is represented by *Sphaerocarpus*. From some *Sphaerocarpus* type, the two main lines of development, the Jüngermanniales and Marchantiales di-



niales, Marchantiales and Sphaerocarpaceae. The gametophyte being Calobryum Blumei, has a prostrate rhizome-like internal with form-developed upright shoots bearing three separate, the gametophyte leaves. The habit is much like that of some acrocarps, and the apical growth is much the same. The living representative of the sexual organs and sporophyte is the acrocarp, existing far antedating any of the other Here it is probable that several families are unrelated to it, and that many of several independent lines of development that may be traced back to a Sphaerocarpus type. In all families of the acrocarps, this tendency to develop definite culminating in the modern Acrogynae. The latter probably do not constitute a single closed phylum but represent a number of end-forms of several independent phyla.

Among the Acrogynae, Fossombronia has been stated as an intermediate form leading up to some of the Acrogynae. On the one hand, Fossombronia shows points of similarity to the Sphaerocarpaceae and, on the other, to Treubia and Petalophyllum, forms with distinct leaves and the tetrahedral apical cell of the Acrogynae. Of the latter group, the Lophoziaaceae, e.g., Nardia and Lophozia, may be distantly related to the Fossombronia series. Another independent line that has been proposed is the Lejeuneaceae, some of which in their early stages show a flat thallus like that of some of the simple Anacrogynae, the Aneuraceae. It must be added that these conclusions are not admitted by many students of the Jungermanniales who regard the Acrogynae as monophyletic.

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## EXPLANATORY NOTES

*By the editors*

Acrogynous: growing at the apex.

Archegoniates: all plants possessing an archegonium, a particular kind of female reproductive organ. Includes liverworts, mosses, ferns and gymnosperms.

Elaters: fine hair-like bodies found among the spores of hepatics, consisting of a single cell with walls spirally thickened. They assist in the dispersal of spores by their hygroscopic movements.—Grout.

Gametophyte: the sex cell- or gamete-bearing phase of a plant. It develops from a spore borne on the sporophyte, *i.e.*, by the spore-bearing phase. In Bryophytes the sporophyte is always parasitic on the gametophyte and connected with it by a so-called 'foot' at the base of the 'seta' which bears the 'capsule.'

Hepaticae: liverworts; together with mosses, the Musci, they comprise the Bryophytes, one of the primary divisions of the Archegoniates.

Homoplasmy: resemblance, but not involving common origin.

Pyrenoid: a structure connected with chloroplasts of certain green algae and apparently associated with starch formation.

Ulothricales: an order of green algae.

## EVOLUTION OF POLLEN GRAINS

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If one examines a random collection of pollen grains, such as might be obtained from a sample of honey (1), the muddy bottom of a pond (10, 14), or from a sticky slide exposed to the winds (23), the forms encountered are surprisingly various, as various, in fact, as the plants which produced them. There may be the one-furrowed form of the magnolia, the smooth single-pored globular grain of grass, or the three-furrowed grains of pea, rose or buckwheat; there may be the minute grains of forget-me-not, so small that they are apt to be entirely overlooked, or the enormous grains of hollyhock and four-o'clocks of several thousand times the bulk; and always there will be the large grains, with their two bladdery wings, of the pines. Some, like those of the grasses, will be smooth, while others, like those of the composites, will be bristling with spines or covered with a reticulum of vertical ridges marking the surface into a geometrical pattern. At first sight, these different forms appear to be entirely unrelated, yet they have all been derived from each other by evolutionary processes quite comparable with those whereby the plants which produced them were derived.

But evolution alone does not give pollen grains their varied forms. In considering pollen grains it is necessary always to bear in mind their rather peculiar mode of formation in tetrads. Nowhere else than among pollen grains are the words of Nägeli (12) truer, when he said: "A correct understanding of a thing can be gained only by a knowledge of its beginning as well as its ending." A correct understanding of pollen grains can be gained only by considering them against a background of their generation. The fact that they originate in tetrads is almost invariably the primary moulding force which gives them their completed form.

Pollen grains are formed from a pollen mother-cell in a way which is almost unique in cell formation. The nucleus nearly always goes through two divisions in rapid succession, followed by two *successive* or *simultaneous* divisions of the cell, forming four

new cells which, by growth and further development, become pollen grains. At maturity, however, they are generally quite separate and it is difficult to see just what the effects of their contacts with each other were. In some few cases, however, the grains never separate, but remain united in tetrads throughout their life; in many others, occasional monstrosities occur, the grains remaining united at maturity in the same relative positions in which they were formed, a sort of "Siamese" quadruplet. We learn from a study of these that pollen grains may be associated with each other in all possible arrangements of four cells in contact, but the two arrangements most commonly observed are: (1) the tetrahedral, in which the grains lie in a compact cluster, so-called because they occupy the relative positions of the angles of a tetrahedron; (2) the tetragonal or flat arrangement, in which they occupy the positions of the four angles of a square or rhombus. The former is a least-surface configuration and results from a postponement of the first division of the pollen mother-cell until after the second division of the nuclei, enabling the four daughter nuclei to slip into the least-surface, or most compact, configuration before the cell walls are formed. The second is the result of two successive divisions—generally bipartition with rectangular intersection, which compels the cells to occupy the positions relative to each other assigned to their nuclei at the time of their division. The simultaneous division (Fig. 1) of the tetrad with its resultant tetrahedral arrangement is prevalent among higher dicotyledons (4, 7), and the successive division is prevalent among primitive dicotyledons, monocotyledons and gymnosperms. But there are so many exceptions that this can scarcely be called a rule. Nevertheless, the way in which the grains are formed determines, in large measure, their ultimate shape.

There are three basic forms of pollen grains or microspores from which, it appears, all others are derived. *First:* There is the form with the triradiate crest, marking the boundaries of the three faces of contact the grain made with its neighbors of the tetrad (Fig. 2A). Each of the radii of the crest is a double ridge which may easily split open, providing lines of dehiscence through which the sporoplast emerges at time of germination. This form is common among the Filicales and primitive gymnosperms, but among angiosperms is known to occur only in the Magnoliaceae. *Secondly:*

There is the one-furrowed or monocolpate form (Fig. 2B). It may be elongate or rounded but is always provided with a single germinal furrow which forms on the side of the grain remote from contact in the tetrad. This furrow serves as a place of emergence for the pollen tube at time of germination. This form characterizes the grains of most gymnosperms, monocotyledons and lower dicotyledons. *Thirdly*: There is the three-furrowed or tricolpate form, provided with three germ pores or three meridionally arranged germinal furrows (Fig. 2C). Each pore or furrow forms at a point of contact that the grain makes with its three neighbors of the tetrad, and one or more of them serves as a place of exit for the pollen tubes. This type of grain characterizes all higher dicotyledons, though it is more often than not profoundly modified, so that it is not easily recognizable. These three basic forms represent three different modes of response to contact stimuli received in the tetrad. In the first, the stimulus results in the flattening of each of the faces of contact with the formation of ridges between them; in the second, no visible effect is produced at sites of contact, but their stimulus forces the formation of the pore or furrow to the opposite side of the grain; in the third, the stimulus induces the formation of a pore or furrow on each of the contact faces. These different modes of response denote huge genetic differences, yet they are not entirely unrelated.

If we examine the most primitive form of pollen grain we find it to be a simple, rounded, protoplasmic body enclosed by a coat in which an inner and outer layer can be distinguished, the outer firm and protective, the inner delicate but impervious; the two layers are thus comparable to the casing and inner tube of an automobile tire. This description would suit a fern spore equally well, which the most primitive pollen grains very closely resembled. Grains of this kind are found among fossil Cycadofilicales, which were not greatly in advance of the ferns. For example, a pollen grain of the Paleozoic *Crossotheca* found by Kidston (9) is virtually the same as a fern spore. It has a triradiate crest on one side, marking off the three faces which were in contact with its three neighbors of the tetrad (Fig. 2A). Its opposite side, which was outward in the tetrad and which for convenience we will call the ventral, was rounded. Such a grain had no germ pore or germinal furrow. Germination here was similar to that of a fern spore; the nucleus

went through several divisions forming a prothallus which, as it grew, ruptured the cell wall, apparently through the triradiate crest and threw it off. We see a similar development in the grain of the fossil *Aetheotesta elliptica* (Fig. 4 *Cycadofilicales*) which was found in its pollen chamber by Renault (15), divested of its outer coat. Then, it is believed, the cells of the prothallus formed motile spermatozoids which were set free in the pollen chamber and brought about fertilization.

This simple pollen grain had no performed germ pore or furrow, but like all other pollen grains and fern spores too, its contents must have had the property of readily taking up and giving off water with consequent increase and decrease in volume, and since this could not be accommodated by any change in shape of the dorsal surface, on account of the stiffening effect of the triradiate crest, changes of volume must have been accommodated by the ventral side of the grain. This we may readily see in such a fern spore as that of *Osmunda* (Fig. 3). When the *Osmunda* spore dries, its contents shrink and a large and deep concavity forms on the ventral surface and, though the spore itself was originally spherical, the concavity is generally elongate. Here, then, is the most primitive germinal furrow, merely a tucking-in of the ventral surface, its position forced there by the presence on the dorsal surface of the triradiate crest or, if the grains of the tetrad failed to separate, by the presence of its three neighbors.

It will be noticed in the foregoing example that the ventral furrow primitively served as a mechanism to accommodate changes in volume and not as a place of exit for a pollen tube; the germination of such fern spores as that of *Osmunda* takes place by dehiscence through the triradiate crest. Moreover, this function of the furrow of accommodating changes in volume has been retained, together with its more recently acquired function of permitting the exit of the pollen tube, among the higher gymnosperms and angiosperms. For this reason I have coined for it the term *harmomegathus* which means an organ of volume-change accommodation.

The next stage in the evolution of pollen grains is perhaps represented by those of *Dolerophyllum* (15, 16) and *Whittleseya* (19), among the Cordaitales (Fig. 4). These are fossil trees which lived in the Mesozoic period and were probably the precursors of our modern conifers. Their pollen grains differed from those of the

Cycadofilicales in having a well defined preformed furrow on the ventral side. It appears that, as the prothallus of these grains developed upon reaching the pollen chamber of the seed they were to fertilize, the invaginated furrow became evaginated and finally separated from the rest of the exine, opening like a lid, and permitted the escape, into the pollen chamber, of motile spermatozoids. Here, then, was a preformed organ which was both a harmomegathus and a germinal furrow; it permitted changes in volume due to changes in moisture; it permitted the growth of the prothallus without prematurely rupturing the spore coats; finally, it split off, and permitted the escape of the fertilizing elements. But in these grains the prothallial tissue was much less extensive than in those of the Cycadofilicales. The reduction of prothallial tissue which marks the evolution of pollen grains all the way up to the angiosperms had already set in.

The next stage in the evolution of pollen grains is perhaps represented by the Bennettitales (Fig. 4). Here prothallial elimination has proceeded much further. Occasionally, the whole grain was partitioned into a few large cells, but more often only a few rounded cells were cut off and pressed against the inside of the pollen wall. With this prothallial reduction, however, there is not found a corresponding reduction in the size of the furrow; it still persists as a wide-open gash reaching from end to end of the grain, yet much less necessary than before (2, 21, 22).

A further step forward is seen in the grains of *Ginkgo* and the Cycadales. Here is found virtually complete prothallial elimination. At germination, a sort of pollen tube is formed; it functions somewhat differently, however, from that of the grains of angiosperms. But the germinal furrow still persists, extending the whole length of the grain (Fig. 4). It has no means of closing on account of its rounded ends and it appears to be enormously larger than necessary, leaving the grain wide open over a large proportion of its ventral surface (8, 17). It may be a significant fact that the four great groups, Cycadales, Bennettitales, Ginkgoales, and Cordaitales, which possessed grains with the wide open furrow, are now either nearly or quite extinct.

This type of grain, since it was common to all the more primitive forms, must have been the natural heritage of higher gymnosperms, such as the conifers, and of angiosperms, whatever their



origins may have been. And as we pass in review the grains of the different groups, I think there can remain no doubt that the effects of this wide-open furrow, which had been developed to meet a need which no longer existed, were, in its new associations, more detrimental than beneficial. The evolution of pollen grains from this stage onward is largely the story of the modification, protection, reduction or elimination of the wide open furrow which was their heritage from the past. The ways in which the different groups disposed of it forms one of the most dramatic chapters in the whole of pollen morphology.

Among conifers we find a number of rather obvious ways in which this was done. Curiously, each way characterizes, for the most part, one or two of the tribes and suggests that each may represent a separate line of development. The Araucarineae, regarded by many as the most primitive of gymnosperms, exhibit, perhaps, the simplest way. The floor of the furrow appears to have been evaginated and its exine thickened. In some, for example, *Agathis* (Fig. 4), the grains are spherical with exine of uniform thickness throughout so that one could not guess that they bore any relation to one-furrowed grains, but in others, for example, *Araucaria* (Fig. 4), a rim-like thickening is quite evident, which can only represent the rim of the all-but-vanished furrow.

The Podocarpineae, also primitive and in some ways similar to the Araucarineae, disposed of the furrow by developing a wing-like bladder around it. This is so arranged that when the grain dries and contracts the furrow dips in, causing the frill to buckle together, tightly closing the gap. The ability to develop this bladder frill was undoubtedly inherited from the remote past. Similar bladders are found on grains of some ancestral Cycadofilicales as, for example, *Stephanospermum caryoides* (Fig. 5). In this case, the frill was developed probably as a floating organ for its possessor lacked a germinal furrow; it was a large grain, however, about a hundred microns in diameter, so that some floating device was necessary to enable it to reach its goal. Among podocarps, however, floatation does not seem to be the primary function of the bladdery wings for the grains are small enough to float unaided; in fact, they are no larger than those of most wind-pollinated angiosperms which manage quite well without any floating devices. Among podocarps, the bladdery frill became an organ of

protection for the furrow. In response to this new function it became modified in two distinct ways. When the furrow was elongate the frill became divided into two halves which close over the furrow like the shells of a clam (Fig. 4, *Podocarpus*). But if the furrow was not elongate the frill became separated into three detached bladders which served the same function (Fig. 4, *Pherosphaera*). It should be noticed, however, that the grains of *Saxegothaea*, which is generally regarded as belonging to this group, are without bladders, resembling, in this respect, the grains of the Araucarineae (20).

Pollen grains with similar bladders are found among the Abietineae, for example, those of *Pinus*, *Abies*, *Picea*, *Cedrus* and *Pseudolarix* (Fig. 4); but it is not a universal character in this tribe either, for bladders are entirely lacking in the grains of *Tsuga*, *Larix* and *Pseudotsuga* (Fig. 4). The presence of bladders on the grains of the Podocarpaceae and Abietineae has suggested to many investigators that the two tribes may be related. In other respects, however, the grains of these two groups are quite different; among podocarps, in those grains which have two bladders the furrow is always sharply defined with a distinct rim to which the ventral roots of the bladders are attached, whereas among the winged grains of the Abietineae the furrow is not sharply defined and has no rim. Furthermore, the grains of the Abietineae are generally about three times as large in their linear dimensions as those of the Podocarpaceae. The possession of bladders seems to be merely one of those characters which appear again and again throughout large groups and immense periods of time; it is even older than the furrow itself.

Another method of dealing with the furrow was by reducing its size. In the grains of *Taxodium* and *Torreya* (Fig. 4) we find it represented by a small and slightly elongate weak spot on the ventral side, which bulges somewhat when moistened but seems too small and ineffective to be of any importance in accommodating changes in volume. In the grains of *Cryptomeria*, *Sequoia* and *Glyptostrobus* (Fig. 4) of the Taxodineae, the furrow is pinched up into a pointed papilla which takes no part whatever in accommodating the grain to changes in volume; when these grains dry and contract the whole ventral surface dips in, saucer-like, with the papilla standing up in the middle. Grains of *Glyptostrobus* are

often united in their tetrads and the little papillae always face outward, showing by their position that they are really homologues of the germinal furrow, though they are so far reduced that they might not otherwise be recognized as such.

A further step in the reduction of the pore is found in the grains of *Cunninghamia* (Fig. 4). Here the papilla is only a vestige, so small and insignificant that it cannot always be seen, if, indeed, it is always present. Total elimination of the furrow is found in the grains of *Taxus*, and in those of *Juniperus* and *Thuja* (Fig. 4); in fact, throughout the Cupressineae.

The question naturally arises: What takes the place in these grains of the vanishing furrow? It had two very necessary functions, germinal emergence and volume-change accommodation, which cannot be dispensed with. As the furrow was progressively reduced, first to a pore, then to a papilla, and then eliminated, there was a progressive reduction in thickness of the exine and increase in thickness of the intine. In the grains of *Juniperus*, which we may take as the culmination of this line of development, the exine is thin and flexible, easily accommodating ordinary changes in volume, and there is an enormously thickened intine which, upon germination, swells, ruptures the exine and throws it off completely, the grain developing thereafter as a naked prothallus—a curious reversion to the ancestral method of the Cycadofilicales.

Still another way of dealing with the furrow was adopted by the grains of *Welwitschia* and *Ephedra* (Fig. 4) which are probably best regarded as advanced gymnosperms. In *Welwitschia* the grains retain their thick exine. The furrow is simply floored over by the thick and inelastic material of the exine, thereby greatly impairing its function of harmomegathy. This function is taken over by a large number of grooves and ridges which enable the grain to change its size and shape without rupturing its walls. The furrow, though quite evident, plays but little part in the adjustment. In the grains of *Ephedra*, which is undoubtedly related to *Welwitschia*, the process of furrow reduction is carried a step further. In those of most species the furrow is entirely absent, its function being taken over by the grooves and ridges with which these grains are still better provided than those of *Welwitschia*; either these are small and numerous, as in the grains of *Ephedra altissima*, or they are large and fewer in number and of a highly specialized character

as, for example, those of *Ephedra glauca* (Fig. 4). The exine of these grains is very thick and inelastic, permitting no stretching, but this is compensated in the grains of *E. glauca* by a thin zigzag streak of elastic material in the bottoms of the grooves, giving off branches outwards toward the crests of the ridges. These permit the grain readily to change its shape by allowing the ridges to become more flattened and broader and at the same time more arched throughout their length as the grain expands, and at time of germination afford lines of dehiscence. In its extraordinarily specialized grain, *Ephedra* seems to stand very high among gymnosperms or represents a highly developed group coordinate with them.

Among gymnosperms we have thus seen a number of different ways of dealing with the wide open furrow which was their heritage from the past. In the Araucarineae it was pushed out and floored over, among the Podocarpineae and Abietineae lateral bladders were developed which fold over it, in the Taxineae it was reduced to a pore, in the Taxodineae to a papilla, and in the Cupressineae completely eliminated. It was simply floored over with its impaired harmomegathic function transferred partly to a large number of longitudinal grooves in *Welwitschia*, and completely eliminated and its functions entirely taken over by longitudinal grooves in the grains of *Ephedra*. As far as evidence of the pollen grains can tell us, these various methods of disposing of the furrow may represent as many lines of development, that is, coordinate branches of the gymnosperm stock. But it does not imply that the Podocarpineae are closely related to the Abietineae because both tribes developed bladders in some of their genera, nor that the Taxineae are related to the Taxodineae because in both of them the furrow is reduced to a papilla. These could as well be duplications of the same method of disposing of the furrow in coordinate groups.

The same primitive single-furrowed type of grain appears also to have given rise, perhaps directly, to those of most monocotyledons. It is true that there is much variation among the grains of this group but, outside of the Alismataceae and a few associated groups which appear to be related to the Ranales, there is nearly always just one furrow or pore or none at all, for all through the group, as among gymnosperms, there is a strong tendency to do away with

the furrow. The grains of the Palmaceae (Fig. 4) are one-furrowed, almost the same as those of primitive gymnosperms; their only advance over those of cycads is an increase in length and the more pointed ends of the furrow, which permit the latter to close tightly. The grains of the Liliaceae and allied families, of Typhaceae, Sparganiaceae, Juncaceae and others, though often highly modified, are likewise clearly derivatives of the one-furrowed type. In the grains of the Musaceae and Cannaceae (Fig. 4, *Canna*) the furrow is completely eliminated with the exine extremely thin and the intine extremely thick, comparable, in this respect, to the grains of *Juniperus*. In the pollen grains of the grasses (Fig. 4, Gramineae) the furrow is reduced to the smallest possible pore, provided with a little lid or operculum which closes it tightly when the grain dries.

The Nymphaeaceae are somewhat anomalous in their position; in some characters they are monocotyledonous, in others dicotyledonous. It is, therefore, interesting to see what their pollen grains tell about them. The pollen grain of *Castalia* (Fig. 4, Nymphaeaceae) is shaped something like a turtle bereft of its appendages, with an upper and lower shell and between them a ring-shaped strip of elastic membrane. When this grain dries the flattened side is drawn inward and when it is moistened it is pushed out again, its free movement being permitted by the thin elastic membrane which surrounds it. The grain of *Nymphaea*, the yellow water-lily, is similar, except that the furrow is elongate and its enclosed area of the exine is a narrow strip which becomes completely tucked in when the grain dries. At first sight one would be tempted to say that these grains have a ring-shaped furrow, entirely different from anything we have yet seen. But if we examine them a little closer we find that the rounded or dorsal surface is generally covered with long spines, while the exine of the ventral surface, which is surrounded by the ring of thin exine, is generally nearly or quite smooth; furthermore, there is often a distinct difference in the texture of the two surfaces showing that the area enclosed by the ring is not just a part of the exine cut off from the rest. A more correct interpretation seems to be that the grain has one large furrow occupying the greater part of its ventral surface, and the detached piece of exine is its operculum, morphologically its thickened furrow floor.

Interpreted in this way, we see in the Nymphaeaceae just another way of dealing with the furrow. The pollen of the Nymphaeaceae, therefore, suggests that the plants may not be either monocotyledons or dicotyledons, but should perhaps be regarded as a coordinate group, possibly derived from the Bennettitales and on a par with the Magnoliaceae which are regarded by some as the direct descendants of the Bennettitales.

The one-furrowed type of grain, while it is characteristic of the gymnosperms and monocotyledons, is not confined to these groups. It reaches a little way up into the dicotyledons in surprising fashion (5, 6). It is found without any important modification in the Saururaceae, Piperaceae (Fig. 4, *Piper*) and Chloranthaceae, which are regarded as among the most primitive members of the dicotyledons, and the possession of this type of grain by these groups is a most remarkable confirmation of the position assigned to them at the beginning of the dicotyledonous series. Outside of these admittedly primitive families, the one-furrowed type of grain is found also in the Magnoliaceae (Fig. 4, *Magnolia*), which is in keeping with the position assigned to them in most modern classifications. But it is not found in the Salicaceae, Juglandaceae, Betulaceae, Casaurinaceae, and other Amentiferae (24) which are also sometimes regarded as primitive. The grains of these families show unmistakable signs of reduction and apparently trace their origin to some of the higher dicotyledons.

The pollen grains of the remaining dicotyledons are entirely different from those of the lower dicotyledons and gymnosperms (24). Instead of having a single furrow or pore forming on the part of the grain most remote from contacts with its neighbors of the tetrad, the basic form of grain of the higher dicotyledons has three pores or furrows which form at the points of contact that the grain makes with its neighbors of the tetrad (Fig. 2C). The organizations of the two types of grain are thus fundamentally different and, for the most part, must represent an enormous genetic gap, but, by the most extraordinary good fortune, we find that gap nicely bridged in the Magnoliaceae.

The Magnoliaceae are generally regarded as consisting of three tribes, Magnolieae, Illiceae and Schizandreae. There is a tendency nowadays, though, to regard the two latter tribes as belonging to a separate family, the Schizandraceae. Judging by their pollen

forms, there still seems to be some relationship between the two families, distant perhaps but significant. Therefore, in this discussion I prefer to retain the older classification. The pollen grains of the tribe Magnolieae are one-furrowed and scarcely to be distinguished from those of the Bennettitales. Wieland (21) says: "The Magnoliaceae must be among, if not the most primitive of all the angiosperms." He regards such species as those of *Magnolia* and *Liriodendron* as directly descended from the Bennettitales—little more than modernized williamsonias or wielandias. The pollen grains of the tribe Magnolieae, in their simple and unmodified single furrow, suggest that they are certainly among the lowest angiosperms, perhaps on a par with the Saururaceae and Piperaceae though not necessarily closely related to them, because their grains are very much larger.

Somewhat similar are the pollen grains of *Drimys* (Fig. 6) of the tribe Illiceae, but their particular interest lies in the fact that they remain united in their tetrads at maturity. Since the tetrads are tetrahedral the grains are rounded or somewhat triangular, and each has a single furrow facing outward, and which is rounded instead of elongate, but it functions in the same way as in the grains of the Magnoliaceae, bulging outward when moistened and dipping inward when dried, and serving as a place of emergence for the pollen tube at time of germination. The main points of interest in this grain lie in the fact that it shows plainly that the position of the single furrow is on the distal side of the grain in the Magnoliaceae in relation to its tetrad, as it is in grains of primitive gymnosperms. That this relation is practically universal among pollen grains was first observed by Fischer (5). I have, therefore, designated it as Fischer's law.

The grain of *Illicium floridanum* (Fig. 7), which belongs to the same tribe as *Drimys*, is quite different. It does not have any trace of the large single furrow. Instead, it has three slender furrows which reach from pole to pole, dividing the exine of the grain into three equal lunes. These three furrows appear not to be homologous with the three furrows of the ordinary dicotyledonous grain. They do not participate in harmomegathy—these grains are thin-walled and shrink by simple collapse in one or more of their lunes—and they do not provide places of emergence for the pollen tubes. Instead, they provide lines of dehiscence along which the exine

splits completely apart into three separate sections which are cast off. In this respect they resemble the triradiate crest of the fern spores with which, it therefore appears, they are homologous, though this point has not yet been settled. If this interpretation is correct we have here a curious survival of an extremely ancient type; it is the only example of dehiscence that I know among pollen grains of angiosperms. It seems a curious paradox that of the grains of *Drimys* and *Illicium*, which are conceded to be closely related, one should possess an ancient pteridosperm feature and the other a gymnosperm feature; the significance of this paradox, however, becomes more apparent when we consider the grains of the next tribe of the family.

In the grains of *Schizandra* (Fig. 8), of the tribe Schizandreae, there are six furrows meridionally arranged. Three of these are long and meet at one pole but end blindly about 45 deg. short of the opposite pole, and three of them are short and do not meet at either pole. These latter alternate with the long furrows crossing the equator by which they are nearly or quite bisected. At the free pole, where no furrows meet, the exine is flexible and may dip in or bulge out in response to changes in volume. The three furrows which meet at the opposite pole provide lines of dehiscence through which the pollen protoplast may emerge; they are the homologues of the triradiate crest of the fern spores and pteridosperm spores. It is, therefore, safe to assume that the pole at which they meet was proximal in the tetrad and that they represent the boundaries of the three faces of contact that the grain made with its neighbors of the tetrad. The three short furrows appear to serve no function, unless they merely stiffen the exine and so limit the flexible area to the region of the distal pole. Nevertheless, their position across the contact faces between the long furrows shows that they are morphologically homologous with the three furrows of the higher dicotyledons. The flexible area around the distal pole corresponds to the single furrow in the grains of gymnosperms. Thus we find combined in this remarkable grain the main features of the pteridosperms, of the higher gymnosperms and of the higher dicotyledons, the three basic types which represent three different responses to contact stimuli in the tetrad.

It is true that the three short furrows of the *Schizandra* pollen grain accommodate neither changes in volume nor pollen-tube emer-



gence as their homologues do in the pollen grains of higher dicotyledons, the former function being provided for by the flexible polar area and the latter by dehiscence through the point of confluence of the three long furrows. Nevertheless, the structure of the three short furrows is exactly the same as that of the long furrows, so it seems reasonable to suppose that, among higher dicotyledons, with loss of the triradiate crest which is entirely absent from their grains, these three short furrows could easily have taken over their function by simply splitting longitudinally and, having once acquired a longitudinal split, they could likewise take over the function of volume-change accommodation. Such appears to be the origin of the three furrows which characterize the pollen grains of higher dicotyledons. In grains of *Schizandra* they function only as structural stiffening of the walls. In grains of higher dicotyledons they represent the taking-over of two additional functions coincidentally with elimination of the organs which formerly performed them.

Furrows of this type belong strictly to pollen grains of higher dicotyledons; they are not found elsewhere. Development of these furrows was the great achievement of the dicotyledonous pollen grain. With it the grain was released from limitations imposed upon it by the single long deep furrow which had been its heritage from ancestral gymnosperms of the remotest antiquity. With this release came the most astonishing diversity of form developed through the relatively short succeeding span in the evolutionary scale, standing in remarkable contrast to the continuous monotony of the preceding development of the one-furrowed grain. Through some such form as that of *Schizandra* the grain appears to have been set free with the acquisition of a new set of organs allowing it a new way of doing things, and of this the enormous variety of pollen forms among higher dicotyledons is the expression.

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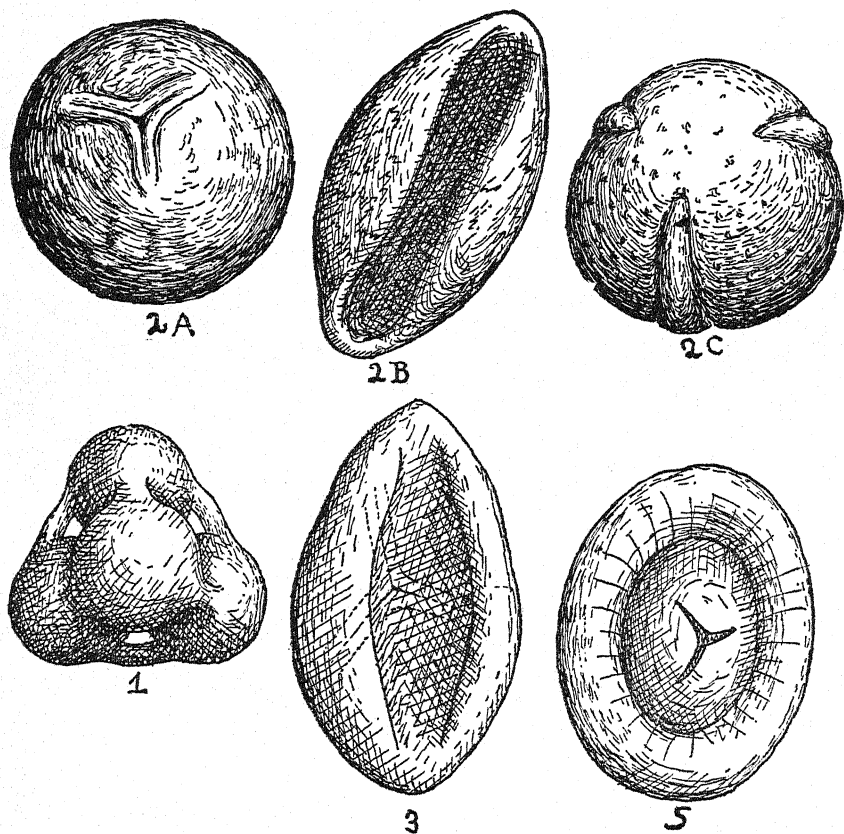


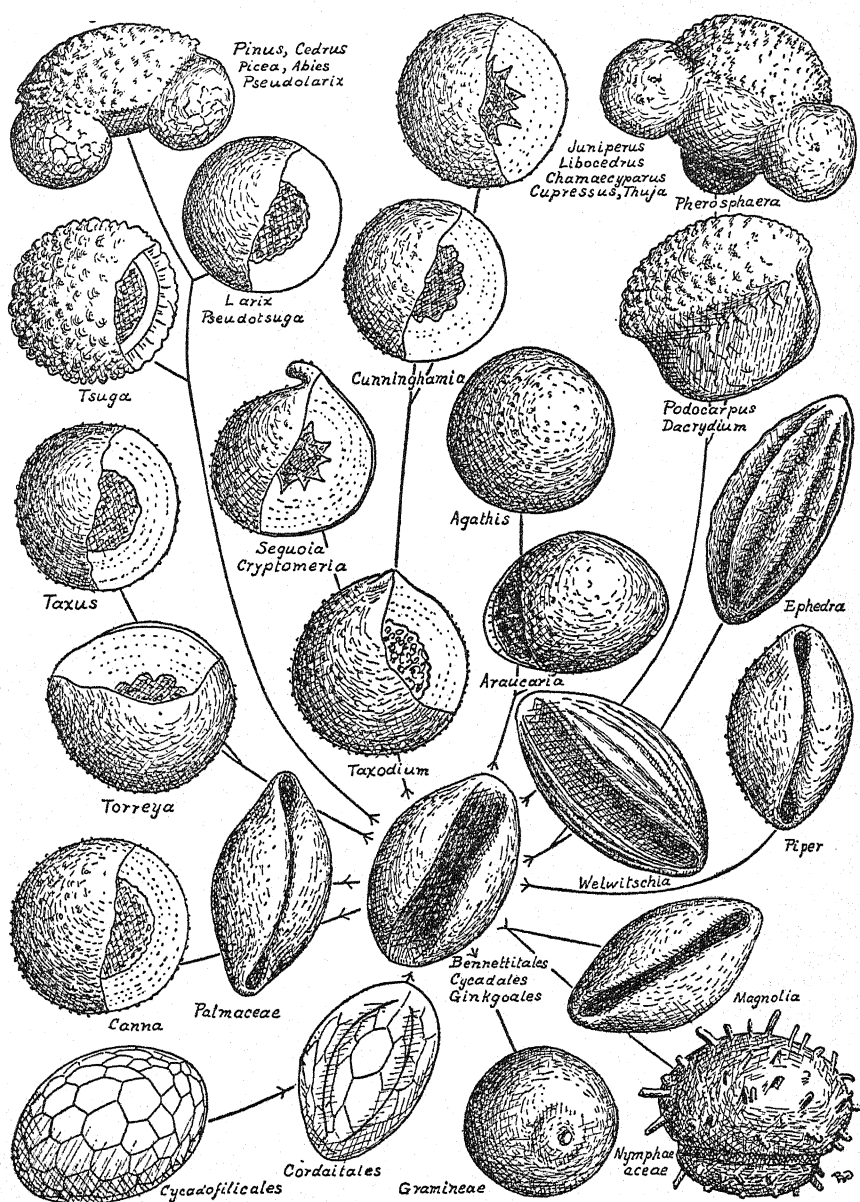
FIG. 1. Pollen tetrad in the tetrahedral arrangement with the four daughter cells about to separate, diagrammatic.

FIG. 2. Three basic forms of microspores and pollen grains; A. with triradiate crest; B. with single furrow on the ventral side, monocolpate; C. with three furrows meridianally arranged, tricolpate.

FIG. 3. *Osmunda* spore, diagrammatic, ventral view with its single temporary furrow uppermost, drawn as if transparent to show the triradiate crest on the dorsal side.

FIG. 4. Representative pollen grains, semi-diagrammatic, showing the sequence in which the various forms might have been derived from each other. Reproduced, with modification, from *Pollen Grains* by the present author (24), with permission of the McGraw-Hill Co., Inc., New York.

FIG. 5. Pollen grain of *Stephanospermum caryoides*. After Oliver (13).



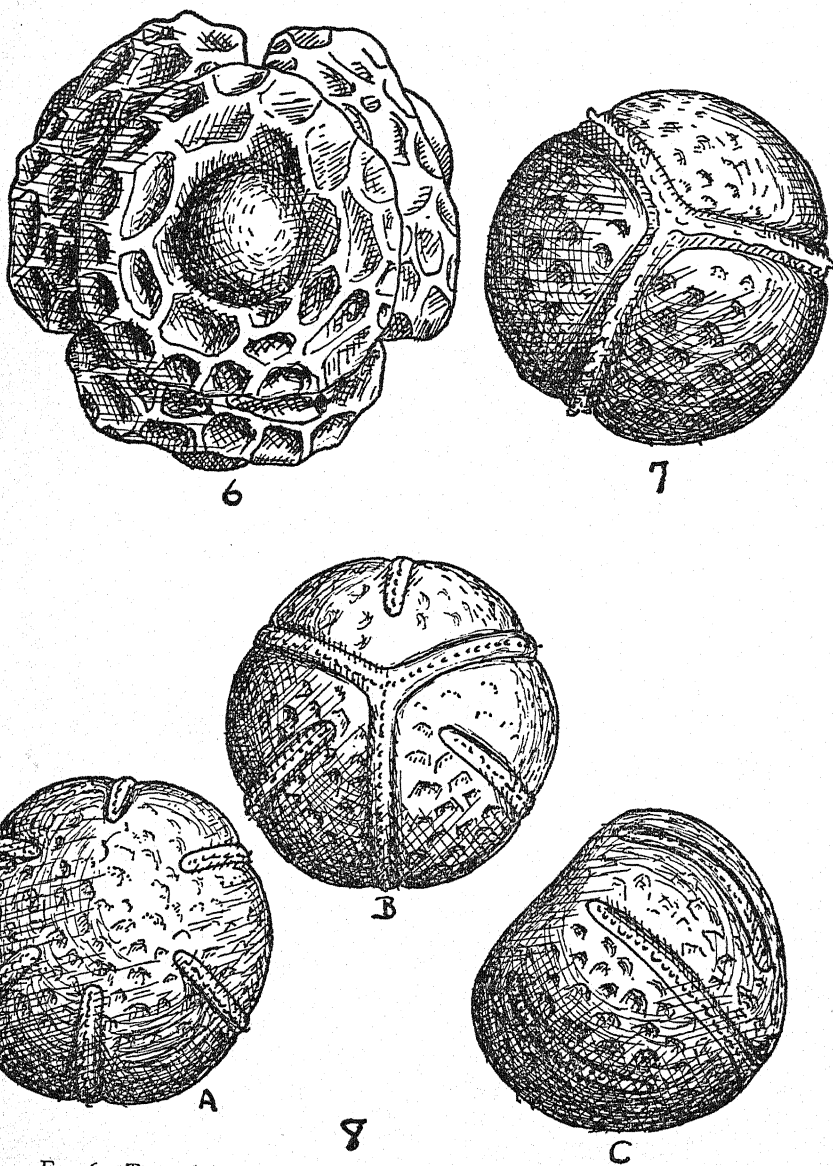


FIG. 6. Tetraglobate pollen of *Drimys Winteri*,  $47\mu$  in diameter.

FIG. 7. Pollen grain of *Illicium floridanum*, polar view,  $28\mu$  in diameter.

FIG. 8. Pollen grain of *Schizandra chinensis*,  $21.6\mu$  in diameter, three views, A. ventral, B. dorsal, C. side.

## CYTOPLASMIC INCLUSIONS OF PHYTOMASTIGODA\*

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### INTRODUCTION

At the present time, there is a certain amount of disagreement as to the nature and identity of the various types of cytoplasmic inclusions in the Phytomastigoda. According to one viewpoint (9), there should be recognized a *vacuome*, consisting of definite vacuoles or smaller inclusions characterized by certain staining reactions, and an *ergastome* composed of "liposomes"; the term *chondriome* is applied only to the so-called initial stages in the development of the vacuome. In another interpretation (20), which is in better accord with the views of the majority of cytologists, the vacuome and the *chondriome* are considered to be entirely distinct types of cytoplasmic inclusions, differing in physico-chemical nature and in staining reactions. As for the identity of the Golgi material in the plant-like flagellates, a number of different views have been expressed. In addition to these more fundamental differences of opinion, the literature contains conflicting reports concerning the identity of the various types of inclusions in specific flagellates.

### MITOCHONDRIA (CHONDRIOME)

The mitochondria of the Phytomastigoda have in most instances been identified on the basis of their reaction to Janus green B in vitally stained preparations, and similar inclusions have been observed in permanent preparations; in a few instances, however, the mitochondria have been identified only in fixed and stained material.

Among the Chrysomonadida, small, relatively numerous granules, stainable vitally with Janus green, have been described as mitochondria in *Chromulina* sp. (23) and similar small inclusions have been stained with hematoxylin after osmic fixation. In *Chilomonas paramecium* (Cryptomonadida) the mitochondria have been reported (23) as granular inclusions similar to those of *Chromu-*

\* The Phytomastigoda constitute a group of flagellate infusorians which possess chlorophyll bodies, plant-like nutrition and a deficient mouth. It is suggested that Sharp's "Introduction to Cytology" be consulted for cytological terms in this article.—Editor.

*lina*. In *Noctiluca scintillans* (Dinoflagellida) both bacilliform and granular mitochondria have been described (6), the former being found in the cytoplasm immediately surrounding food vacuoles and the more numerous spherical mitochondria distributed throughout the cytoplasm.

In *Euglena gracilis* spherical mitochondria, relatively few in number, have been reported (5) in permanent preparations. Larger spherical bodies, identified as pyrenoids, are regarded by Causey as being derived probably from mitochondria. In another investigation (24) both rod-like and granular inclusions have been described as mitochondria in the same species. Brown (3)) found only spherical mitochondria in permanent preparations of *E. gracilis*, and Baker (1) likewise reported numerous granular mitochondria scattered throughout the cell. In *Colacium vesiculosum* (28) small rod-like bodies, mostly peripheral in distribution, are demonstrable with Janus green and have been stained with hematoxylin after fixation in Champy's fluid. In *Astasia* sp. (23) numerous small granules, scattered through the cytoplasm, have been stained vitally with Janus green and demonstrated with hematoxylin in permanent preparations after osmic fixatives. *Menoidium incurvum* (24) shows both granular and bacilliform inclusions when stained vitally with Janus green. In *Entosiphon sulcatum* (30) the mitochondria are relatively small rods, peripherally located and staining faintly with Janus green and also with hematoxylin after fixation by Benda's method. In *Peranema trichophorum* (22) numerous elongated subcuticular inclusions arranged in spiral rows are demonstrable vitally with Janus green and by suitable methods of fixation and staining. The mitochondria of the same species have also been identified (4), in fixed and stained material, as inclusions ranging from small spheres to large discoid structures, whereas Grassé and Poisson (18) have described granular mitochondria in specimens stained vitally with Janus green.

In the Euglenida, in particular, it seems probable that the so-called 'mucus-bodies' described by certain workers have occasionally been identified as mitochondria by some investigators, as well as "une simple modification du vacuome ordinaire" by others (8). Thus, inclusions similar in distribution to the peripheral "mitochondria" of *Peranema* (22) and *Colacium* (28), have been described as mucus-bodies in *Euglena granulata*, *E. proxima* and *E.*

*viridis* (12), and in *E. intermedia* (18). These inclusions are more or less rod-like in *E. proxima* and *E. intermedia*, but are small and granular in *E. viridis*. The mucus-bodies are said (18) to be stainable vitally with neutral red, and are reported (12) as showing an acid reaction in comparison with the more nearly alkaline reaction of the vacuome. In preparations of *Euglena* stained with mixtures of neutral red and Janus green, the mucus-bodies as well as the vacuome are found (18) to be stained with neutral red. In *Peranema* (22), on the other hand, the comparable subcuticular inclusions were stained by Janus green and not neutral red in similar preparations, while the elements of the vacuome were stained with the latter dye. These mucus-globules are said to contribute to the formation of the gelatinous membrane characteristic of the resting stages of Euglenidae. In *Euglena intermedia* (18) each mucus-body is said to lie in a small sac which opens through a fine canal to the outer surface of the organism. In addition to the cases recorded in Euglenida, an 'appareil mucifere' has been described in *Oxyrrhis marina* (10).

In *Polytoma uvella* (Phytomonadida) mitochondria in the form of short rods, and occasionally granules, have been reported (34) as being scattered through the cytoplasm. In *Chlamydomonas* sp. (25) the mitochondria are evident as granules and rods, most of them apparently lying near the surface of the cell. In *Haematococcus pluvialis* (14) numerous small inclusions, usually bacilli-form and mostly peripheral in location, are stained vitally with Janus green.

#### VACUOME

The vacuome of the Phytomastigoda consists of vacuoles or smaller globules or granules stainable vitally with neutral red, brilliant cresyl blue, and certain other dyes, and may be distinguished from the chondriome by staining with a mixture of neutral red and Janus green. It has been pointed out (8) that there is a striking contrast between the vacuome of the great majority of plants and that of the lower organisms. In the latter the vacuome is usually in the form of small globules or granules rather than vacuoles in the strict sense, whereas the reverse is true of the higher plants. These smaller elements of the vacuome have been designated variously as chromatic granules, metachromatic



granules, volutin granules, fuchsinophile granules, and also as 'chromidies' (8). In sealed-slide preparations the vacuome may often be blackened with osmic acid under direct observation after being stained previously with neutral red (22, 23, 24, 25), the process of impregnation requiring several days. Furthermore, the vacuome is consistently impregnated by the usual osmic and silver Golgi methods without previous staining with vital dyes. In some species it has been possible to observe similar inclusions in the living unstained organism, and in neutral-red preparations to follow the gradual staining of these same inclusions. On the basis of present evidence, therefore, it seems that in the *Phytomastigoda*, as in plants in general (21), the vacuome consists of normally pre-formed inclusions which may be stained vitally with neutral red and other vital dyes and may be impregnated by the osmic and silver Golgi techniques.

In *Chromulina* sp. (23) a number of small globules are stainable vitally with neutral red, brilliant cresyl blue and neutral violet. After vital staining with neutral red, the globules may be impregnated with osmic acid under direct observation. Similar inclusions are impregnated in the Mann-Kopsch (Weigl) osmic technique. In *Chromulina maxima* (10) the vacuome consists of relatively few globules, which may be almost completely absent in some specimens. In this case the volume of the vacuome is reduced, as compared with that in many other species of flagellates. In *Synura uvella* (10) the vacuome is stained with extreme difficulty with brilliant cresyl blue, and is usually not stained with neutral red. In *Chilomonas paramecium* (23) the vacuome is represented by globules somewhat larger and less numerous than the mitochondria, but similar to the latter in distribution. The vacuome of *Oxyrrhis marina* (10) consists of scattered globules, said to contain 'metachromatin.'

In *Euglena viridis* numerous small scattered granules have been identified both as vacuome (8) and as mucus-bodies (12). A similar situation exists in regard to *Euglena velata*, in which rod-like inclusions have been identified as "aspect en bâtonnet du vacuome" (8) and as 'mucus-bodies' (12). Grassé and Poisson (18), however, have pointed out that the vacuome and the 'mucus-bodies' are two distinct types of inclusions. The vacuome of *Euglena proxima* (15) consists of small scattered globules, stain-

able vitally with neutral red and reacting to osmic impregnation. Similar inclusions have been described (24) as the vacuome in *Euglena gracilis*. The 'pseudochondriome' recognized by Brown (4) in this species may possibly correspond to the vacuome reported by other workers. According to Baker (1) the vacuome of *E. gracilis* shows a "light orange reaction to intra-vital neutral red." Similar orange granules were observed by Hall (24) in unstained specimens and were interpreted as cytoplasmic pigment granules. It seems possible, therefore, that Baker may have mistaken the orange pigment granules for elements of the vacuome in his preparations. The vacuome of *Colacium vesiculosum* (28) consists of small globules scattered through the cytoplasm, and in *Astasia* sp. (23) similar inclusions are stainable vitally with neutral red, brilliant cresyl blue and neutral violet. Globular inclusions of the same type have been identified (24) as the vacuome in *Menoidium incurvum*. In *Peranema trichophorum* (22) the vacuome is represented by numerous small globules scattered through the cytoplasm.

The vacuome of *Polytoma uvella* (34) is composed of metachromatic granules stainable vitally with neutral red, brilliant cresyl blue and Nile blue. The inclusions are demonstrable also by methods for staining metachromatic granules and they are impregnated by the usual osmic and silver Golgi methods. In *Chlamydomonas variabilis* (8) the vacuome may be represented either by numerous small globules ('chromidies') or by larger 'vacuoles ordinaires.' Hall and Nigrelli (25) reported in *Chlamydomonas* sp. the fusion of small elements of the vacuome to form larger globules approaching in size the 'vacuoles ordinaires' described by Dangeard. The vacuome of *Haematococcus pluvialis* (14) includes a number of globules scattered irregularly throughout the cytoplasm. A vacuome similar to that of other Phytomonadida has also been reported (11) in *Gonium*, *Eudorina* and *Volvox*.

It has been pointed out (19, 21) that the vacuome in plants serves as a center for the accumulation of various products of metabolism, especially those soluble in water, and thus should not be considered a part of the living substance but more properly one of the components of the paraplast, or deutoplast. It might readily be assumed that the vacuome in the plant-like flagellates plays some such rôle in cell activities. This view is supported by

the positive reaction of the vacuome in *Chlamydomonas* (25) to the iodine test for starch.

#### GOLGI MATERIAL

Some years ago Bowen (2) stated, concerning the Golgi material of Protozoa, that with so many divergent opinions "no basis for what may or may not be Golgi material has yet been agreed upon." This statement still holds, since various types of inclusions have been identified by different workers as Golgi material of Protozoa. Several investigators have been tempted to recognize the vacuome of the plant-like flagellates as Golgi material. Others have been equally confident that the stigma, the contractile vacuole, or other specialized organelles should be considered Golgi material.

The similarities of the vacuome to Golgi material have been pointed out in a number of flagellates. In *Chromulina* sp. (23) small globules are blackened in osmic impregnation and resist bleaching with hydrogen peroxide; these inclusions resemble in size, shape and distribution the elements of the vacuome, and presumably are identical with the latter. *Chilomonas paramecium* (23) shows similar osmiophilic globules scattered through the cytoplasm; these likewise appear to be identical with the vacuome. In *Euglena gracilis* (24) small, scattered globules (vacuome) are impregnated consistently by osmic and silver methods, while the stigma is impregnated only occasionally and is much less resistant to bleaching after osmic impregnation than are the elements of the vacuome. Baker (1) has described the Golgi material of *E. gracilis* as follows: "These globules are spherical, oval or ring-shaped and invariably show an irregular inner surface of the periphery. They are quite similar in size to the globules making up the vacuome (volutin) which can be recognized in the same cells alongside these Golgi bodies as light-brown globules. The latter never take the osmic acid other than to appear as light vacuoles even in unbleached organisms. . . . It is believed that those globules which retain the black rim and inner gray center after Kolatchev's method with bleaching, are Golgi bodies and that these bodies are separate and distinct from the vacuome." It is not entirely certain, however, that Baker has succeeded in distinguishing between the vacuome and the scattered orange pigment granules

commonly observed in *Euglena gracilis*. In *Colacium vesiculosum* (28) the elements of the vacuome are consistently blackened in silver impregnation. *Astasia* sp. (24) shows small osmiophilic globules which appear to be identical with the vacuome; likewise, in *Menoidium incurvum* (24) both osmic and silver impregnation methods demonstrate small, scattered globules similar in size and distribution to the elements of the vacuome. In osmic impregnation of *Peranema trichophorum* (22) numerous small globules, apparently identical with the vacuome, are blackened consistently. In *Chlamydomonas* sp. (25) osmic and silver impregnation reveals a variable number of blackened globules, similar in relative size, number and distribution to the inclusions stained vitally with neutral red. In *Polytoma uvella* (34) the usual osmic and silver Golgi methods impregnate the elements of the vacuome, but not the contractile vacuole or the parabasal bodies. In *Haematococcus pluvialis* (14) scattered globules, apparently the vacuome, are blackened in osmic and silver impregnation.

In addition to the vacuome, various other organelles and inclusions have been designated Golgi material. Nasonov (32) maintained that the contractile vacuole of *Chilomonas paramecium* should be regarded as the homologue of the metazoan Golgi apparatus. In the dinoflagellate, *Polykrikos schwartzi* (7), clusters of elongated osmiophilic vesicles have been described around each centrosome; these inclusions have been considered Golgi material. Grassé (13, 15, 16, 17, 18) considers the stigma of *Euglena* homologous with the parabasal body of other flagellates and the equivalent of the Golgi apparatus. This interpretation has been questioned, particularly by Mangenot (31). Brown (3) has pictured the Golgi apparatus of *Peranema trichophorum* as "a network of long, interwoven fibres which are concentrated in the posterior portion of the animal. This network is not so dense in the later division stages of *Peranema* as it is during the early prophase." This 'Golgi network' described by Brown appears to be nothing more than the blackened pellicular striations of the flagellate, previously described (27) in various species as the 'silverline system' of Euglenida.

In attempting to recognize 'Golgi material' in Protozoa, it seems reasonable to look for inclusions with characteristics somewhat as follows: (1) consistently impregnated by the osmic methods, rather

than occasionally impregnated; (2) resistant to the usual methods of bleaching after osmication; (3) consistently impregnated by the silver methods; (4) except for possible specialized types, perhaps similar in general form in different Protozoa; (5) occurrence in Protozoa generally, and not merely in certain species or groups. If such criteria are applied to certain examples of so-called 'Golgi apparatus', the basis for their identification as Golgi material seems rather inadequate. On the other hand, the observations of a number of workers show that the elements of the vacuome more nearly satisfy such requirements than do any of the other types of inclusions or cell organelles previously designated as Golgi material. Hence, it would seem that recognition of the vacuome of flagellates as 'Golgi material' is at least as logical as attempting to identify such specialized structures as the stigma of *Euglena*, the parabasal body of certain flagellates, the contractile vacuole, or other organelles as homologues of the Golgi apparatus. However, this view is opposed by various workers who insist that, even in the green flagellates, the vacuome cannot be homologized with Golgi material. At present, therefore, the identity of the Golgi material in the Phytomastigoda is uncertain.

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# THE BOTANICAL REVIEW

VOL. II

MARCH, 1936

No. 3

## THE RÔLE OF LIGHT IN THE LIFE OF PLANTS

### II. THE INFLUENCE OF LIGHT UPON GROWTH AND DIFFERENTIATION

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#### GROWTH

The relation of light to the physical and chemical constitution of plants, as discussed in the first part, would lead one to expect a profound influence upon growth and production of morphological characters. Preliminary to further discussion concerning the action of light upon processes involved in *growth* and *differentiation*, it may be well to define what is meant by these terms.

*Growth* may be defined as irreversible increase in the size of an organism or its parts as a result of the incorporation of materials from the environment; new protoplasm is synthesized, cellular boundaries are extended and weight is increased. The final results of the integrated processes of growth may be measured in terms of weight, volume, length, number, etc. Growth may be accomplished in one or both of two different ways: (a) cell multiplication and limited cell enlargement and (b) cell enlargement which is unlimited within a comparatively wide range. In order to dis-

tinguish clearly between these two methods of growth (*cf.* 231) the term *meresis* has been proposed to signify increase in cell number by meristematic activity, promoted perhaps through the agency of "meristins" (263) or "biotin" (247); and the name *auxesis* has been applied to growth by continued cell enlargement, activated by growth-substances or "auxins" (247). Increase in size by repeated cell divisions necessitates synthesis of new protoplasm and, in consequence, there results a gain in dry weight by the accumulation of carbohydrates, fats, proteins, etc. Following cell divisions, considerable increase in volume may be accomplished through further cell enlargement brought about by the processes of hydration, vacuolation, or extensive polarized growth, which need not be accompanied necessarily by appreciable increment in solid matter. The metabolic processes leading to formation of new protoplasm and new cells are conditioned mainly by the supply of organic nutrients to growing centers or meristems. The more obvious manifestations of growth by cell enlargement are concerned chiefly with absorption of water and plastic extension of the cell walls. Increasing hydration is usually accompanied by vacuolation, and frequently by changes in the composition of the wall, so that this phase of growth may be looked upon also as a step in the direction of differentiation. In view of the studies made by Priestley (410), Schuepp (478), and others, it appears that even the vacuolated type of cell may synthesize protoplasm and undergo divisions, thus contributing to growth through increase in cell number as well as in cell size. The production of form and structure in plants will be discussed later under the heading *differentiation*.

#### LIGHT AND DARKNESS

It has been emphasized by recent workers that light is important for meristematic activity in green plants through photosynthesis of organic foods, absorption of mineral matter, and regulation of the water supply. If other conditions are favorable, photosynthesis should proceed unchecked in continuous light and the food available for growth might be expected to vary with the exposure period. It has been found, however, that the length of the daily light period not only affects the quantity of material formed, but also influences the use which the plant can make of it. In those plants which store food in tubers, vegetative growth is best in long

daily light exposure periods. In somewhat shorter periods these same plants store carbohydrates, but in very short periods the total light energy becomes a limiting factor in  $\text{CO}_2$  assimilation. In bulb-forming plants, like the onion, growth is favored in days shorter than the optimum for food storage (146). There appears to be an optimal light/darkness ratio for the maximum utilization of organic materials for stem growth. Light seems to exert a "regulatory action on the internal processes of the plant other than those which merely determine the total quantity of carbohydrate produced," and it has been suggested that this regulation by light may be concerned with internal water supply, *i.e.*, the degree of hydration of the living cell contents (146).

The action of visible radiation upon growing organs is striking when comparisons are made between the rates of enlargement in darkness and in light of different intensity values. From the time of DeCandolle (*cf.* 373) and of Sachs (452) many instances are on record to show that growth fluctuates greatly during the natural alternation of day and night. Thus, bamboo grows more rapidly at night provided the temperature permits a just comparison (403, *cf.* 320), and corn behaves in like manner (183). The same behavior has been reported by Mason (303) for the date palm whose organs cease elongation in full sunlight but resume growth slowly in diffused light and rapidly at night. Growth of the palm leaves was apparently correlated with closing of the stomata, checking of transpiration and increased turgesence in the meristematic tissues.

Brown and Trelease (59) found that shoots of *Cestrum nocturnum* decreased in length due to water loss by transpiration during the day but recovered their original size later in the afternoon and increased in length by rapid growth at night. Trelease (549) observed a more rapid rate of elongation in banana leaves at night associated with greater turgidity as compared with conditions in the daytime.

In some plants, such as succulent cacti, the rate of growth during day and night varies according to the acidity of the tissues. In the daylight, greater growth of *Opuntia* sp. appears to be synchronous with decreased acidity, while at night or in dim light there is a lessened growth rate along with accumulation of acids (*cf.* 544). Popp (401) grew soy beans under controlled conditions in a series

of light intensities ranging from 26 to 4285 f. c. and observed that the lower the light intensity the more rapid was the rate of stem elongation during the period of initial growth. Reid (436) observed that illuminated seedlings grown in an atmosphere lacking in  $\text{CO}_2$  were shorter and weighed less than similar seedlings grown in darkness. However, seedlings grown in light (minus  $\text{CO}_2$ ) had larger leaves and cotyledons than those grown in darkness.

The remarkable and abnormal differences in the form, structure and color of plants grown in continuous darkness have been observed and described so frequently that the term "etiolation" has come to have a well-known significance. From the present viewpoint, the great increase in length of the shoot axis in darkness is particularly interesting in those instances where an ample supply of food for growth is available in the storage reserves of seeds, tubers, rhizomes, etc. When such plants are grown in darkness, the internodes are long, the leaves fail to expand and are devoid of chlorophyll, and the root system is poorly developed. According to Figdor (125), etiolated shoots of *Bowiea volubilis* have elongated internodes in the main axis, and branching is very much reduced.

Andrews (6) reported that *Vicia faba*, growing in the light, reached a stem height of 5 cm. and a diameter of 7 mm. in 12 days, but in the dark a height of 30 cm. and a diameter of  $\pm .75$  mm. was attained in the same time. Overbeek (374) reported a growth retardation of over 50 per cent in illuminated *Raphanus* seedlings as compared with control plants kept in darkness. Skutch (501) found that the leaf sheaths of banana became abnormally elongated when placed in darkness for some time. Priestley and Ewing (413) and Priestley (407) have described the structure and morphological responses exhibited by growing seedlings of *Vicia* and *Pisum* when in total darkness and in a series of daily light exposures varying from one minute up to several hours. Light exposures tended to remove the symptoms of etiolation by shortening the internodes, expanding the leaf laminae and causing chlorophyll formation. Trumpf (550) carried out some novel experiments to test the effect of daily exposures to light of different colors upon the growth of *Phaseolus multiflorus*. Using an arc lamp and short exposures of 1, 5, 10 and 30 minutes it was possible to obtain plants which tended to appear more nearly normal

in size and form without development of chlorophyll. Blue rays prevented stem elongation more than did red wave lengths of light. Similar evidence has been obtained by Teodoresco (533) and Funke (138), both of whom grew many kinds of plants with available stored food under different colored glass filters. Blue radiation seemed to cause short petioles and short internodes, while red light, acting in a manner similar to darkness, permitted their excessive elongation. Lange (264) has demonstrated that exposure of germinating *Avena* seed to bright daylight during the period of swelling inhibits any appreciable elongation of the first internode ("mesocotyl") in the seedling. Even exposure to phototropically inactive red light during the swelling period, and for 12-16 hours after emergence of the coleoptile, almost completely inhibited elongation of the first internode. Boysen-Jensen (51) and others have observed how the coleoptile of *Avena* elongates more in darkness than in light. Hamada (181) found that the effect of light upon growth of the *Avena* seedling varied with its age. The first internode was stunted by light, while growth of the primary leaf was stimulated.

Coupin (85) carried out some interesting experiments with *Lupinus* seedlings grown in light and in darkness. Extracts from the light grown plants, when applied to the nutrient solution of seedlings kept in darkness, prevented excessive elongation of the internodes so characteristic of ordinary etiolated plants. Coupin concluded that the action of light on the chloroplasts caused the formation of some substance which prevented etiolation. Trumpf (551) repeated these experiments with *Phaseolus* seedlings and found that the sugar in the expressed sap was responsible for the effects observed on stem elongation, and that the sap of both light and dark grown plants hindered growth of seedlings in darkness when the expressed juice was added to the cultures.

The effects of light and darkness upon growth and development of plants may be summarized briefly as follows: In the complete absence of light, plants with available stored food become etiolated. If a little light is available, the extreme symptoms of etiolation are modified, *i.e.*, the leaves unfold and develop chlorophyll, and the internodes tend to grow somewhat shorter. With further increase in light intensity up to about 20 to 50 per cent of ordinary daylight, the size of the leaves and height of the plant attain a maximum.

Exposure to full sunlight brings about a slight decrease in height, in length of internode and in area of leaves, but these effects are associated with an increase in percentage of dry weight, in number of branches, in size of roots, and frequently also in flowers and fruit (*cf.* 492).

#### CELL MULTIPLICATION

In considering the fundamental nature of the light- and dark-growth phenomena recorded in the literature, it is very difficult to conclude whether observed increases in size may have resulted from increases in the number, or in the size of the constituent cells, or both. In too few instances have determinations been made concerning the behavior of the cells in growing organs, but it seems fairly certain that meristematic activity probably plays a greater part in the phenomenon of increased organ stretching under reduced light conditions (as already described) than is generally believed. Etiolation experiments, designed to compare relative growth and development in darkness and in light, have been conducted usually with growing tubers, seeds, spores, etc., which possess an abundance of food. Since the food supply is of proportionately greater importance for the processes of cell multiplication than for the enlargement of cells through increased hydrolysis, the nutritive factor must be taken into account for the correct interpretation of data given in light-growth experiments.

Interesting facts concerning cell multiplication have been derived from the growth behavior of unicellular green algae. It has been found in *Scenedesmus costulatus* (56), growing in a mineral salts solution, that the rate of increase in growth is approximately proportional to the rate of increase in light intensity until a certain optimum illumination is reached. In experimental cultures grown under different intensities of light covering a considerable range, the addition of glucose to the culture medium did not increase the growth rate beyond that normally found under autotrophic conditions associated with optimal light intensities. When glucose was supplied in the complete absence of light, the shape of the growth line was much less steep than under the conditions of optimal autotrophism in inorganic solutions or partial saprophytism in glucose solutions in the light. Increased light intensity has been reported to shorten the intervals of time between cell divisions



(135), in developing zoospores of *Oedogonium plurivale*. An accelerating effect upon protoplasmic synthesis through carbohydrate supply has been suggested to account for this increased division rate.

Several investigators have found a periodicity in the occurrence of cell division in root tips. The results, though not entirely in agreement, indicate that there are often about two division maxima during a 24 hour period, one of which occurs about midday (cf. 488). Diffuse light has been reported as favorable to continued growth of excised root tips in nutrient culture (439). Droogelever (107) has observed rhythmical division in *Allium* with an alternating maximum and minimum in constant darkness. Illumination during the daytime changed the rhythm to a diurnal period with the largest number of divisions taking place in darkness. Some possible relation of light to nuclear processes has been suggested by the optical sensitization of root tips with dilute dyes (406) whereby abnormal mitoses and retarded growth were obtained (cf. 328). Satisfactory information concerning the effects of light and other factors upon mitosis is indeed meagre.

In considering the action of light upon growth in lower organisms, a recent report from the field of bacteriology is of interest. Fraps and Sterges (134) determined the rates of oxidation of three different salts of ammonia in six soil types, and found that while 41 per cent of the ammonia was oxidized in darkness, only 5 per cent was changed in sunlight during the same time. Apparently, sunlight was injurious to the growth of the nitrifying organisms. Recent work by Meier (315) has indicated that multiplication of the unicellular green alga, *Stichococcus bacillaris*, is proportional to the intensity of illumination ranging from 3.76 to 34.1 microwatts/mm<sup>2</sup>. A higher intensity such as 102.0 microwatts/mm<sup>2</sup> checked the growth of this alga. Some portions of the spectrum were not favorable but a wide complex of wave lengths from 0.6 to 1.4 microns was moderately effective in promoting multiplication of the algae. The cell multiplication of diatoms is reported also as being dependent upon the energy of the incident radiation, probably due to the nutritive relationships (517). Cell multiplication in *Volvox* and *Closterium* has been found to be greater in red than in blue light, (242) probably also because of



the relative efficiency for  $\text{CO}_2$  assimilation. Experiments by Teodoresco (533) and others concerning the number and size of cells in the thalloid bodies of cryptogams grown in the light and darkness have given striking results. When spores were germinated in blue or white light, the number of cells in the sporeling was greater than when grown in red light or in darkness. In regard to the effect of light upon bacteria, it may be said that visible light acting for a long time has an inhibiting effect upon their multiplication, while ultra-violet radiation exerts a lethal action dependent upon its absorption and energy (599).

In multicellular higher green plants, it is more difficult to evaluate the effect of light upon growth by cell number due to mutual contact and pressure of the cell units one upon the other. The peculiar etiolation effects obtained when herbaceous plants are grown in darkness have been explained by Priestley on the basis of the availability of soluble foods to the meristematic tissues. Due to the superficial position of the apical shoot meristem, any variation in its food and water supply would certainly be reflected in its growth behavior (383). The size and number of epidermal cells were determined in etiolated and normal plants of *Phaseolus multiflorus* by Brotherton and Bartlett (58). Increase in length of the etiolated stem was estimated as due 34% to increased cell divisions and the other 66% was accounted for by increase in length of the constituent cells. Penfound (386), who grew *Helianthus* under different conditions of light and soil moisture, found that the increased height of shade-grown plants was due to an increase in the number of cells, not increase in length of cells, along the vertical axis. The repressing effect of light upon sprouting in potato tubers stored either in daylight or in artificial light has been shown to be very marked as compared with controls kept in darkness (574).

In the growth of storage organs, seeds and vegetative parts of the plant body, light appears to play an important rôle through the processes of nutrition and hydration and perhaps photocatalysis. Whether light plays any important part in the production and activity of substances which have been considered by certain investigators to be specific for cell divisions such as glutathione (182), Wuchsstoff B (4), biotin (247) and bios (324), is not known.

## CELL ENLARGEMENT

The effect of light upon the enlargement of organs and tissues has been shown by several different investigators to be concerned, to a considerable extent, with growth of the individual cell-components. Long ago, Kraus (255) investigated the matter of cell size and number in etiolated and normal leaves and stems of several species of plants. The epidermal cells of etiolated internodes were nearly always longer than those in normal plants, and sometimes an increase in cell length was accompanied also by increase in the number of cells. The characteristic elongation of stems in darkness appears to be the result chiefly of a considerable increase in the mean length of the component cells. Redington (430) found that long light exposure resulted in decreased cell formation and cell elongation in the stems of *Kleinia* and *Linum*. The growth period and final size of leaves were greater in 16 hours of light and 8 hours of dark per day than in continuous light. In 8 hours of light alternating with 16 hours of darkness there resulted very long petioles and small laminae. Measurements of endodermal cell length in the stems of flax, hemp, hop, salvia, hibiscus and cotton indicated that the linear dimension varied inversely with the light period (429). Doroshenko (104) reported a reduction in cell-size of wheat, barley and flax when subjected to a moderately short day; however, further decrease in the daily light exposure brought about an increase in length of the cells. On the other hand, Jeffs (224) found no effect of light upon the elongation of root hairs in *Raphanus sativus* or in *Sinapis alba*.

Küster (260) has shown that the cells of etiolated shoots are larger and less differentiated than those of normal light growing plants. Brotherton and Bartlett (58), as has already been mentioned, reported that about two-thirds of the increased length of the etiolated stem may be due to increase in cell length. Klebs (cf. 533) found that the growth and form of fern prothallia depended upon the quality of the light, red rays permitting an increase in length through cell stretching and blue-violet rays causing shorter forms with more cells. Weston (584) has reported that urediniospores of *Puccinia graminis* failed to produce germ tubes in strong light, but Voorhees (563) has found that diffuse light promoted germination of spores of *Physoderma zea-maydis*.

In a long paper dealing with the "influence morphogenique" of different wave lengths of light, Teodoresco (533) emphasized the need for controlling wave length and energy value of the radiation and the necessity of divorcing the trophic action of food supply from the blastic effects of light. Working with tubers, rhizomes,

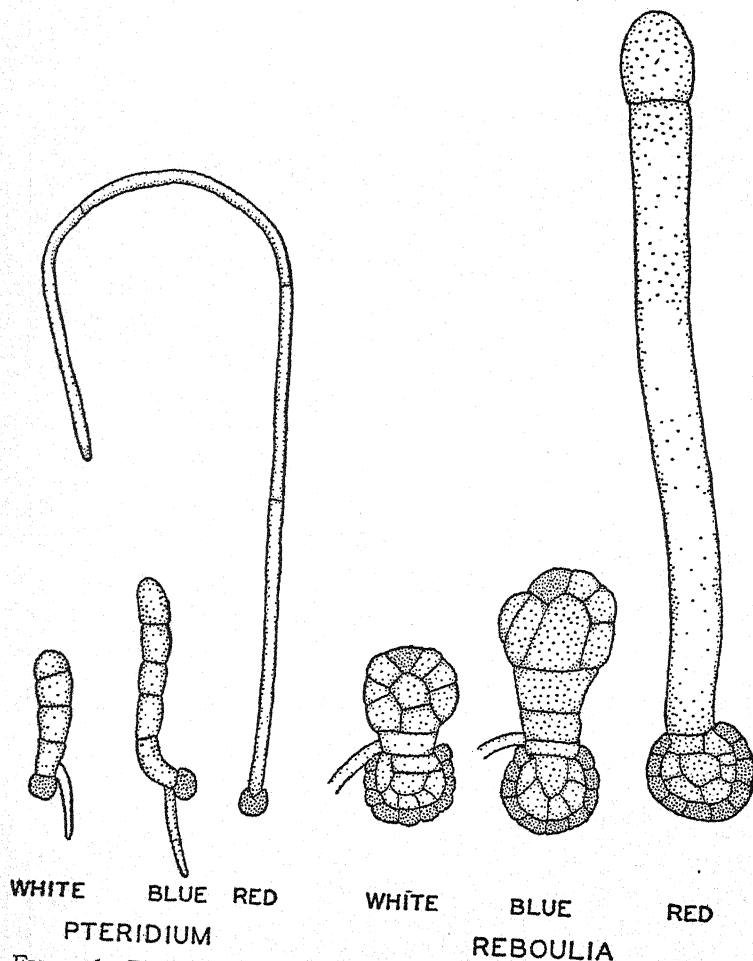


FIGURE d. The formative effect of light upon fern (*Pteridium aquilinum*) and liverwort (*Reboulia hemisphaerica*) sporelings. Plants germinated in white or in blue light are stout and multicellular, while those grown in red light or in darkness tend to become slender and few celled. After Teodoresco (533).

seeds and spores, he was able to show in a convincing manner that darkness and red light permit excessive tissue and cell elongation and that blue light exerts a stunting effect upon growth by keeping the cells smaller. His figures of fern spores germinating in dilute Knop's solution and 1% agar illustrate the striking differences between the very slender few celled filaments under red light or in darkness and the short many celled protonemata which tend soon to broaden out into typical gametophytes in blue or white light. Blue light retards both spore germination and cell elongation. That the tendency for increased cell elongation in darkness is in no way concerned with chlorophyll mechanism is further emphasized by Brefeld's (*cf.* 533) observation of the behavior of the fungus *Pilobolus* whose sporangiophores elongate more in darkness than in light. Results similar to those of Teodoresco have been reported also by Klebs (*cf.* 291) and by Stephan (521) and Hommer (206) for young fern prothallia. Exposure of the germinating spores to red light resulted in a narrow prothallium with a terminal meristem; in the blue light the prothallium became shorter and broader, with a lateral meristem. Gistel (161) observed that the normal form of *Schistostega* protonemata was attained only in the presence of air and light. Schmid (471) found that liverwort spores germinated only in the light. In *Preissia* the cross walls increased in frequency and the cell length decreased with increasing intensity of light. The protonemata of *Lophocolea* and *Chiloscyphus* were single filaments when grown in weak light but became much branched under a strong Osram lamp.

In view of the accumulated facts dealing with the effect of light and darkness upon growth, it seems fairly clear that the linear dimension of organs and their constituent cells tends to be reduced by the incidence of short wave lengths of visible radiation in the blue-violet region of the spectrum. When growth takes place in darkness or in light devoid of the blue-violet rays, polarized growth becomes accentuated. The length of cells in etiolating organs is known to increase manyfold, but little is known concerning the actual changes brought about in the volume of these cells. Recent discoveries concerning the rôle of electric potential and special growth-substances appear to be of great significance in relation to the problem of polarized growth.

Some earlier statements in the literature relative to the axial orientation of germinating eggs of algae in relation to light (392, 589) may be interpreted now on the basis of electrical polarity and differential distribution of formative materials in the cells. Nienburg (354), as well as certain earlier investigators, has reported that light induced polarity in *Fucus* eggs so that the rhizoids grew out from the shaded side. Goebel (163) observed that the radial podetia of the lichen *Cladonia* may become dorsiventral under the influence of one-sided lighting. According to the same author, the young prothallia of *Osmunda* grow transversely to the direction of light, but *Ceratopteris* and *Adiantum* grow toward light at first and later assume a transverse position. The rhizoids of *Osmunda* and *Preissia* are negatively phototropic. Wakeman-Bonne (564) has observed that the spindle during cell division of *Eremosphaera viridis* orients at right angles to the rays of light.

Light growth reaction has been studied in the *Avena* coleoptile by many investigators (101, 577, etc.). Van Dillewijn (101) illuminated coleoptiles by means of three laterally placed mirrors and found that following sudden lighting there appeared a growth depression. Moreover, it was shown that different regions of the coleoptile differ in their responses to light (cf. 52, 506). Haig (179) obtained similar information which suggested that two different photochemical systems may be responsible for the differences between the "tip response" and the "base response" in the *Avena* coleoptile (cf. 506).

Many fruitful investigations of light growth response of the single celled sporangiophore of the fungus *Phycomyces* have been made in relatively recent years. Tollenaar and Castle (cf. 69) have found that when light is suddenly cut off from a light adapted sporangiophore, there occurs a temporary decrease in its growth rate (the "dark-growth response") followed by a gradual return to about the previous rate of growth in the light. Castle (67) has found, further, that when a sporangiophore which is light-adapted and growing at a constant rate is exposed to more intense illumination, a temporary acceleration of growth (the "light-growth" response) is produced, followed by a decrease in rate until the original rate of growth is regained. The reaction time for the light-growth response conformed with the Bunsen-Roscoe law,

provided the time component of stimulating was small, but when exposure was longer than a few seconds, the reaction time to light was determined by the intensity and not by the energy of the flash (70). In general, the sporangiophore reaches a final height which is greater the lower the intensity of light in which it is grown (70). When actively growing sporangiophores, adapted to a series of light intensities covering a wide range, were suddenly darkened and the times required for the onset of the "dark-growth" response (*i.e.*, reaction time) were measured, it was found that the rate of dark adaptation was proportional to the logarithm of the preceding light intensity (69). The kinetics of dark adaptation has been explained as a bimolecular reaction, as are all other photosensory systems which have been examined by different investigators (67). The reaction times of the "dark-growth" response and of the "light-growth" response are compound, consisting of an exposure period and a latent period, this comprising both the true latent period resulting from photochemical action and any "action time" necessary for the response (67, 69).

Development of the fruiting body of at least some mushrooms is dependent upon light, and abnormal forms appear in complete darkness. Boriss (45) has shown that the wave lengths most effective in bringing about normal expansion of the fruit body in *Coprinus* are those between 400–500 m $\mu$ , *i.e.*, the same region which is phototropically active. Furthermore, the linear growth rate in cultures of *Sclerotinia* has been found to be greatest in alternating light and darkness than in continuous darkness (180).

To explain the mechanism by which short waves of light exert a depressing effect upon the growth in length of plant axes has not been easy, and only in very recent years have investigators been successful in making an approach toward a satisfactory solution of the problem. The increase in size of the growing vacuolated cell has been regarded primarily as a matter of progressive hydration resulting from the activity of osmotic substances in the protoplast. According to Stiles (525), cells of etiolated plants have been found to possess lower osmotic values than those of normal plants, and intense illumination usually has been associated with increase in osmotic pressure. However, total sugars, nitrogen and ash have been found higher in etiolated shoots of *Berberis*

(479). Hence, the osmotic concentration alone does not easily account for the cell size response to light conditions. Recently, it has been claimed by Montemartini (331) that the shorter wave lengths of light diminish the power of attraction of protoplasm for water. That light brings about increase in permeability of the plasma membrane to various solutes has been claimed by many investigators (cf. 53, 528), but Ruhland (449) reported no measurable influence of light upon permeability of beet leaf cells to sugars. Elasticity of cell walls has been found to decrease upon exposure to ultra-violet rays (156). It appears that no final judgment can yet be given concerning the rôle of light in relation to osmotic phenomena and cell size increase.

The study of phototropism, *i.e.*, the growth curvature of an organ in response to unilateral illumination, has made promising advancement toward an understanding of light and growth phenomena. (See (65), (373), and (52) for a discussion of, and many references to the literature of phototropism). By the use of physical methods for measuring intensity of light and response of the plant, Blaauw (31, 32) was able to show a relationship between the energy of the light stimulus and the amount of tropistic response in accordance with the Bunsen-Roscoe law. It was held that light acted by hindering growth and that each part of a plant grew at independent rates according to the amount of light which it received (32). Hence, phototropic curvatures must be due to the difference between the light growth reactions on the two sides of the unilaterally illuminated organ. The inadequateness of this theory as an explanation of light growth phenomena has been brought out by subsequent investigations.

#### GROWTH-SUBSTANCES

A series of experiments by Boysen-Jensen, Paal, Söding and others (cf. 52) led to the idea that there probably exist special substances which regulate growth. In 1928 F. W. Went (577) published the results of important quantitative experiments which confirmed the anticipated existence of a plant growth-hormone controlling the enlargement of the coleoptile of *Avena* seedlings. Furthermore, it was shown that light affected the distribution and activity of the hormone in a definite manner in the plant. Among the many recent papers on the subject, that by Overbeek (374)

affords a good discussion concerning the movement of the growth-substance and its activity in the presence of light in *Raphanus* seedlings. It was found that the production of growth-substance was greatly reduced in the dark but that the response of the *Raphanus* hypocotyl to the hormone was greater in the dark than in the light, the growth response being measured either by total elongation or by growth curvature. The decrease in the ability of the cells to respond to the action of growth-substances (Blaauw effect) is explained, in theory, by the effect of absorbed light quanta upon the electrical charge of intermicellar substance which hold the cellulose pattern in a more rigid position in light than in darkness. (See 5). The hormone was found to move in a polar manner toward the shaded side when the seedlings were unilaterally illuminated so that an unequal distribution of the growth-substance resulted. The effects of different wave lengths of light upon cell enlargement have been investigated indirectly in studies of phototropism, and definite spectrum sensitivity curves have been plotted by various independent workers. In general, the blue-violet has been found to retard growth on the light side of unilaterally illuminated organs much more than the red region (cf. 68, 228) of the spectrum. These results of phototropic investigations which deal with localized growth are in general agreement with results obtained when entire plants are subjected to the action of selected wave lengths of radiation.

A discussion of the interesting paper by Thimann and Skoog (536) dealing with functions of the growth-substance in *Vicia faba* may serve to illustrate further the action of light through the growth-hormone. To test the relation of light to the production of growth-substance, comparable seedlings of *Vicia faba* were placed in darkness and in light for four days. At the expiration of this period the terminal buds were removed and tested for the concentration of the hormone by measuring the curvatures produced in *Avena* coleoptiles under standard conditions. Considerably greater amounts of the growth-substance were found in the light exposed plants. Furthermore, a known amount of growth-substance in small agar blocks was placed on the decapitated apex of each of twelve plants, and pure agar without growth-substance was added to twelve other decapitated plants as controls. After four days in the dark it was found that the plants to which growth-substance



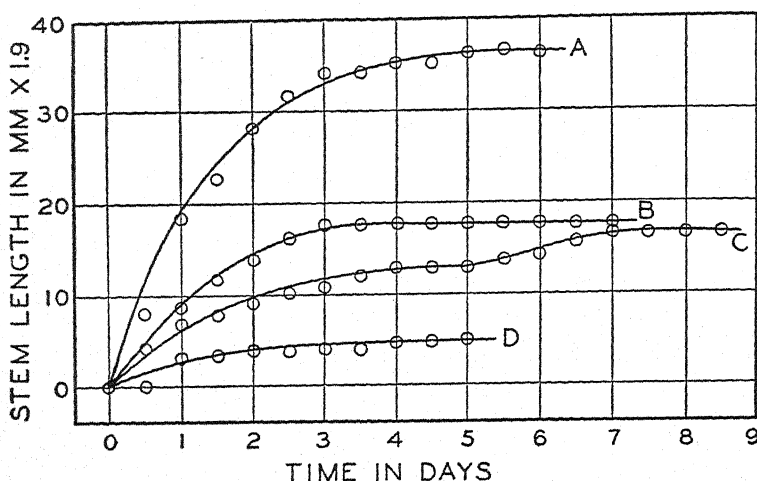


FIGURE e. The formation and action of growth substance in *Vicia faba*. Selected young plants were decapitated and agar blocks with and without growth-substance were applied to their apices. The stem lengths were measured every 12 hours in plants kept in the light and in darkness. Curve "A" represents the rapid growth of plants treated with a known amount of pure growth-substance and kept in complete darkness. The plants of curve "B," having the same quantity of growth-substance added but kept in the light, showed less growth. No growth-substance was added to series "C" which represents growth in the light, nor to "D" which represents growth in darkness. Growth-substance appears to be synthesized in the presence of light (as suggested by the late rise in the growth curve "C"), and exerts its greatest effect in darkness (curve "A"). After Thimann and Skoog (536).

had been added had grown twice as much as the controls. Hence, the growth-substance appears to be made in the light and is capable of powerful action in the dark. Another experiment by Thimann and Skoog was performed as follows: Selected plants were decapitated, all buds and leaves were removed, and the cut surfaces were covered with paraffin. One half of this set of plants was placed in darkness and the other half was kept in the light. Agar blocks, each with 1600 units of pure crystalline growth-substance, were applied to the apices of half the plants in each of the two series in light and darkness, and agar blocks without growth-substance were placed on the others as controls. Every twelve hours the stem lengths were measured. Those plants kept in the light and with growth-substance grew more rapidly than the controls and reached a constant length in 3-4 days. The controls continued to grow steadily and in seven days closely approached the growth

rate of plants with growth-substance. This continued growth increment may be explained as a response to continued synthesis of growth-substance in the stems of plants kept in the light. The controls in the darkened series showed little growth but the plants to which growth-substance in agar blocks had been applied exhibited very great growth. It was concluded that though growth-substance is produced in the light, it functions better in the dark. It may be seen that when plants are brought from the light into darkness, the stored growth-substance acts with greater effect to give a positive dark growth response.

Although the specific and ultimate way in which the plant growth-hormones influence cell enlargement is not definitely known as yet, their discovery in the plant kingdom has created a new approach to the problems of morphogenesis which seems destined to become an exceedingly fruitful field in modern plant science.

#### GROWTH OF THE PLANT AS A WHOLE

Whether growth of the organism as a whole is considered as irreversible increase in total size or as the transformation of unorganized external material into the organized substance of the entire plant (431), the processes involved appear to be capable of rational interpretation. Biologists, with mathematical inclination, have proposed various and sundry formulae in attempting to express the orderliness of organic growth. Blackman (34) long ago pointed out that the amount of material added in growth is dependent upon the amount already present. He likened the ways of growth to the compound interest law, and stated that the obvious measure of the plant's activity is the rate of interest. This rate of increase (*i.e.*, the efficiency index) which is a result of the growth processes, varies, with the genetic constitution and the environmental circumstances of the individual. By some plants, the elements of the environment are synthesized into living matter at a rapid pace; in others, the rate of assimilation is slow. At any given time the significant rate of growth must be expressed as growth relative to the size of the organism (219). Furthermore, there is considerable evidence to suggest that light influences the course of plant development in other ways than through the supplies of food and moisture.

## LIGHT INTENSITY

Using duckweed as illustrative material, Blackman (35) has shown how the growth rate may be accelerated with increasing light intensity up to about 1000 f. c. Above this rather low light value, the rate of frond production is not increased. Many horticultural plants show better growth at higher intensities (*cf.* 597). For example, the tomato gives an increasing gain in dry weight because of photosynthesis proportional to the intensity up to about 4500 f. c. (35, *cf.* 40). That light may have a retarding effect upon the growth rate of certain vegetative organs at the same time, may be seen from Blackman's plot of the increase in leaf area of tomato plants exposed to different intensities up to about 6000 f. c. In unit time, the rate of leaf expansion varies inversely with the intensity of the incident light. In general, it has been found that weeds are able to grow more vigorously and reproduce better under shade conditions than crop plants (597).

Popp grew four different varieties of soy beans under six different light intensities, averaging 4285, 1536, 560, 390, 250 and 25 f. c. in cloth compartments constructed inside a glass house. Weekly measurements of height and general observations of leaf development, thickness of stems, time of flowering, vigor, etc. were made for seven weeks during the course of the experiment. The rate of stem elongation in the initial period of growth varied inversely with the light intensity, while stem thickness was proportional to the intensity. In general, those plants receiving the greatest amount of light were the most vigorous and produced the best leaves and fruit. With decreasing light intensity, there was a gradual decrease in vigor, to the extent that plants grown under 26 f. c. were etiolated and survived only three to four weeks. Using height as a criterion, it was stated that growth of all plants except those under the weakest light "followed the general curve of a monomolecular autocatalytic reaction." The more active phase of the growth curves appeared to be associated with the development of nutritional independence brought about by the ascendancy of photosynthesis, while the later decrease in growth rate appeared to be caused by development of flowers and fruit. This is in accord with the fact pointed out by Murneek (344).

Bolas (40) performed some instructive experiments to determine the influence of light and temperature on the assimilation

rate of seedling tomato plants. The relative increase in dry weight of young tomato plants during seven hour periods was determined over the temperature range 45–90° F. for each of four different intensities of light—100, 200, 600, and 1000 f. c. At the low intensity of 100 f. c., which might be found in a greenhouse on a dull winter day, the assimilation rate rose with temperature from 45° F. to about 62° F., but above this temperature the rate fell off and higher temperatures were harmful to the plants. At 600 f. c., representing a bright spring day, the maximum rate of assimilation occurred at 75° F., and on a bright day with 1000 f. c. intensity, a temperature of 90° F. was not excessive from the point of view of assimilation rate. Assimilation rates were plotted also for each of three different temperatures, 60°, 75° and 85° F., over a range of intensity up to 1000 f. c. At 60° F. maximum assimilation occurred at about 200 f. c. and then decreased considerably with greater illumination. At 75° F. maximum assimilation was at about 750 f. c., the rate falling off toward 1000 f. c. and at 85° F. the greatest assimilation was afforded by the higher intensities in the region of about 1000 f. c. Emphasis was given to the fact that practically all energy required by the green plant for synthesis of organic materials out of  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and inorganic salts, must be obtained by light absorption. For a given leaf area with a certain chlorophyll content (*cf.* 128) there appears to be a maximum assimilation rate for any particular light intensity, and for each intensity there is only one temperature at which the assimilatory mechanism of the plant works most efficiently.

Davis and Hoagland (95) grew wheat plants in controlled environmental conditions with various intensities of light and with different daily exposure times. When the yields were computed it was found that tissue formation is more efficient when the radiant energy is distributed at a moderate intensity over a longer time than under the opposite conditions of high intensity and short duration. For any given illumination value there exists a critical temperature, because of the effect of temperature on the rate of  $\text{CO}_2$  reduction in the light and the rate of oxidation of the organic materials in both light and darkness. Harvey (188) found that potato and tomato grew well under artificial electric light at 380 lumens per square foot. They were taller than usual at this intensity, but gave a more normal appearance at 680 lumens.

Ashby (14) found that growth of *Lemna* increased with intensity up to 700 f. c. and decreased at 1400 f. c. due partly to a decrease in the amount of chlorophyll and partly to some other process. Under intensities of 350, 700 and 1400 f. c., most rapid growth occurred in continuous light and least in the six hour daily light period. Miss Hicks (201) found that the frond number in *Lemna* colonies grown in the range 15° to 30° C. and under 350 to 1400 f. c. increased exponentially for the period of the experiments. Between 15° and 22.5° C. the increase in relative growth rate with temperature was independent of the light intensity, but at higher temperatures growth varied definitely with light intensity which was probably a limiting factor here. The real rate of carbon assimilation in *Lemna* appeared to be about six times the respiration rate at 25° C. Within the range of exponential growth, the final frond area was independent of light intensity, but the frond weight increased with light.

Ashby and Oxley (16) have made an analysis of the influence of light intensity and temperature on rates of assimilation and of frond multiplication in *Lemna*. The increase in rate of frond multiplication with light intensity was significant up to about 750 f. c., but the net assimilation rate, calculated on a basis of the increment in dry weight, increased linearly with light intensity over the whole range examined. The effect of light was brought about apparently through some other process than assimilation. It was found that a wide range of multiplication rates could occur at any one assimilation rate, and that frond area was independent of temperatures above 24° C. and of light above 350 f. c. The total area of a colony was found to depend upon temperature and light only in so far as these factors determined the rate at which new primordia were formed.

Steinbauer (519) grew seedlings of *Fraxinus pennsylvanicum* under low light intensities with controlled temperature and humidity, and varied the concentration of nutrients to test the effect of nutrient supply upon the length of the period of survival at sub-normal intensities. A greater response to an increase in nutrients was found at higher intensities, indicating greater synthesis, but the minimum light requirement could not be lowered by increasing the amount of available nutrients. Shirley (491), Bates (21) and others have studied the effect of light intensity upon growth

of coniferous seedlings for which light is an important ecological factor. The rôle of light in plant succession has been explained on the basis of intensity as a factor limiting increase in dry weight. In general, those species whose light requirement for survival is comparatively little are able to exist for a long time in the shade, and when favorable light conditions are presented, these tolerant seedlings, already well established, are in a position to replace their intolerant competitors (492).

Clements and Long (81) grew sunflower in a series of varying soil moisture (holard—13, 18, 26, 35 per cent) and for each holard the following light intensities were employed—8, 16, 32 and 100 per cent of normal sunlight. It was found that water assumes the major rôle in stem elongation, and light the larger part in production of dry matter. Shirley (490) investigated the influence of light intensity upon growth of buckwheat, sunflower, tomato, tobacco, California redwood, loblolly pine, etc. The plants were grown in soil under a series of different intensities of sunlight varied by using cloth shades, and with temperature and humidity controlled within limits. Determinations of the dry matter, growth in height, chlorophyll, leaf area, etc., were made. The light requirement for survival of these plants was very low, being less than 40 f. c. for all except sunflower which required a much higher intensity. At low light intensities the dry weight production was almost directly proportional to the intensity up to about 20% of full summer sunlight, but at higher intensities the curve sloped off. Percentage of dry matter in the tops, ratio of dry weight of the roots to dry weight of the shoots, vigor of growth, strength of stem and leaf thickness all increased with increasing light intensity. Above 50% intensity the amount of growth increased very little with further increase in light, which agrees with results of most other investigators (401, 597). The sunflower was able to use light for increment in dry matter up to the highest intensities used, but *Geum* and hog peanut showed a decided decrease in efficiency at the greater intensities. According to Lundegardh (291), the assimilation rate of shade plants, such as *Oxalis*, shows a marked decrease in efficiency at about 1/10 full sunlight, while in typical sun plants, such as *Nasturtium*, the assimilation rate increases with light over a much greater intensity range. Over a range of light intensity, all assimilation curves rise more rapidly at lower intensi-

ties than at higher, the particular form of the curve being dependent upon the kind of plant and other operative factors. Lundegårdh (291) has stated that the effect of unit increase of light intensity upon assimilation is greater, the nearer light approaches to a "minimum factor."

#### LIGHT DURATION

Some effects of varying daily light period upon growth and maturation of plants were known even in the times of John Ray (1686) and Linnaeus (1732), according to Laurie (266) and Smith (504). Since the discovery of photoperiodism in recent years, a vast amount of research has been done in an attempt to formulate the principles of growth and development in relation to duration of light. In 1920 Garner and Allard (145) published the results of their experiments in growing many kinds of plants under various lengths of daily illumination. The action of the light period in initiating or suppressing sexual reproduction in various flowering plants indicated that some plants are more sensitive than others to the length of day factor. In the more sensitive group, some are caused to flower more quickly in long days, others in short days. To test a plant for its photoperiodic type, one needs only to grow it under daily exposures of ten hours and eighteen hours per diem, and observe under which, if any, daily light period flower formation is accelerated. Quoting from Garner (144): "When exposed to a day length in excess of the critical, there is in the short-day type pronounced long continued elongation of the stem without flowering; while exposure to a day length shorter than the critical quickly initiates reproductive activity. On the other hand, in the long-day plant, exposure to a length of day in excess of the critical results in elongation of the axis, which is promptly followed by flowering, while exposure to a length of day below the critical tends to limit development to a leaf-rosette stage."

Both types of plants were grown by Garner and Allard (148) under short alternations of light and darkness, extending down to those as short as 5 seconds. In all the plants used, as the alternations of light and darkness were progressively shortened, increasing evidences of chlorosis, impaired nutrition and decreased growth culminated in alternations of about one minute, but with shorter

alternations of 15 and 5 seconds there was a pronounced improvement in the nutrition and growth of the plants. "Increase of the light interval to twice the interval of darkness in each of the cycles had the effect of improving the general appearance and in some cases seemed largely to overcome the retarding action of these alternations on growth" (149). These growth responses to short alternations of light and darkness are the more interesting in view of increased photosynthetic efficiency under conditions of rapid flashes of light which has been obtained in experiments by Emerson and Arnold (119). It is not impossible that these effects of "flashing" light on growth are merely outward manifestations of internal photochemical effects upon specific growth-substances.

Lubimenko and Szeglova (285, 286) have given considerable attention to photoperiodic adaptation and growth as measured by the increase in dry matter per hour of illumination. Maximum utilization of light for growth was attained at a shorter daily exposure than was useful for maximum production of dry substance by photosynthesis. The physiological distinction between short- and long-day plants was ascribed to differences in the enzymatic, apparatus of the cells concerned in the processes of oxidation (respiration) and reduction (assimilation).

Arthur, Guthrie and Newell (12) carried out an elaborate series of experiments with many kinds of plants grown under artificial climates at the Boyce Thompson Institute, where light and  $\text{CO}_2$  supply could be controlled on a large scale. Cabbage plants were found to increase in total weight and in carbohydrate content with length of day up to 17 or 19 hours. Clover, soy bean and cucumber increased in carbohydrates and weight per plant with additional  $\text{CO}_2$  and supplementary light supplied at night by a battery of 1000 watt lamps. Potatoes grew well on long days but formed tubers under continuous illumination only at comparatively low temperatures ( $68^\circ \text{F.}$ ). Barley and spring wheat, in contrast to potatoes, grew and yielded well at a high temperature ( $78^\circ \text{F.}$ ) when given additional light and  $\text{CO}_2$ . The weight per plant of barley was found to increase with day length up to 19 hours per diem. Roses, sweet peas, snapdragons, petunias and nasturtiums grew and flowered well with additional light, but geranium, coleus and tomato were greatly injured by continuous artificial illumination. Good growth of tomatoes has since been reported under



outdoor conditions in Alaska where the sunlight effective for growth was practically continuous (92).

Pfeiffer (390) reported that when buckwheat was grown in 5, 7, 12, 17, 19 and 24 hours of artificial light daily, a maximum height and stem diameter was obtained in the 17 hour day. Smith (504) found that maximum growth, expressed as dry weight per day per unit of light, occurred in continuous light for young plants, but as the plants aged the maximum was displaced toward the shorter day lengths. Adams (1, 2) found that plants exposed longest to the action of light generally gave the greatest average weight and height. In the case of tomato, soy bean, buckwheat and hemp, there appeared to be an upper limit of light duration above which no additional growth was made. Cotton has been found to increase its height with increasing day length up to continuous illumination under the experimental conditions which were employed by Berkley (26).

The relation of light to growth vigor is of interest in view of certain recent investigations of heterosis in plants, where the  $F_1$  hybrids show similar efficiency indices but exhibit different degrees of vegetative vigor (15). Malinowski (301) investigated the effect of photoperiods upon hybrid vigor in *Phaseolus vulgaris*. Two distinct strains were crossed and the hybrids grown under long and under short daily light exposures. In long days the  $F_1$  plants exceeded the parents in height, number and length of internodes and size of leaves. In 8-hour days the  $F_1$  plants were reduced to approximately the parental size and flowering occurred six weeks earlier than in the long day condition. It would be highly instructive to know the effects of light duration upon the efficiency index for growth in plants such as these.

#### LIGHT QUALITY

The visible spectrum has been shown to exert marked effects upon growth through carbohydrate synthesis and through a special formative action not fully understood, while the infra-red region appears to be active mainly through its temperature effects. The great bulk of literature dealing with effects of ultra-violet radiation upon plants has been reviewed recently by Popp and Brown (402). It seems clear that the short wave ultra-violet from 289–200 micro  $\mu$  is distinctly harmful, the degree of injury depending upon the

intensity, the wave length and the amount absorbed by the vital tissues (*cf.* 317, 402, 7, 136, 137). This lethal radiation given off by the sun is filtered out by the ozone in the outer layers of the atmosphere, and hence never reaches the earth. Perhaps it is not surprising that artificially produced wave lengths shorter than ordinary sunlight should bring about unusual effects upon organisms which have become adapted to the natural light environment of which these lethal rays form no part. Popp and Brown (402), after a critical survey of the botanical literature, state that even in very slight doses the short wave ultraviolet has never been demonstrated to be beneficial, and evidence from the most accurately controlled experiments to date shows little, if any, beneficial effect of that region of the ultra-violet present in sunlight.

The action of different portions of the visible spectrum upon size and form of plants has received attention from many investigators. Popp (401) grew a number of different species in colored glass houses using filtered sunlight as the source of energy. Very little difference was noted between plants grown under full sunlight and those grown in the absence of ultra-violet radiation. When the blue end of the spectrum, including all wave lengths shorter than 529 micro  $\mu$ , was excluded, growth was poor, the plants were weak and there was a decrease in fresh and dry weight and an increase in the moisture content resembling the symptoms associated with etiolation. Unfortunately, the intensities were not balanced in the different houses but the data indicate that the blue-violet end of the spectrum is indispensable for normal vigorous growth of plants. Somewhat similar experiments by Shirley (490) have indicated that the blue-violet part of the solar spectrum is more efficient in dry weight production than the red end, when the intensities are 10 per cent of the outside sunlight. Pfeiffer (390) reported better development, as expressed by greater stem thickness, height, leaf thickness, etc., in the full solar spectrum than in any fraction of it. Roodenburg (444) found in his experiments with light from neon and mercury arcs, that the blue end of the spectrum tended to make plants grow stocky. Shirley (490) grew plants in five different colored glass houses with three different intensities controlled by cloth shades in each house. The complete solar spectrum was considered more efficient for the production of dry matter per unit of light intensity than any portion of it.

A further report by Arthur (8) showed that without blue light, plant stems were thin and weak and the leaves small and rolled. When grown without red light, the plants possessed a normal appearance, but growth in height was subnormal due to the low intensity of energy available under the filter glass. Later investigations by Arthur and Steward (11) have suggested that a relatively high proportion of infra-red in the incident radiation may bring about increased elongation of the stems, accompanied by decreased expansion and diminished chlorophyll content of the leaves of buckwheat plants. It was concluded that the efficiency of various gaseous discharge lamps and Mazda lamps in the production of dry plant matter was not related to their emission spectra and the absorption bands of chlorophyll.

Experiments dealing with the influence of near infra-red radiation on plant growth and coloration have been described by Johnston (227) who has pointed out the need for taking into consideration the presence of infra-red energy in order to properly evaluate the effects of the visible region. Marglobe tomato plants were grown under two different distributions of radiation of equal visual intensity, one limited entirely to visible radiation, the other including a large amount of near infra-red energy. After two weeks, the plants exposed to visible plus infra-red radiation were characterized by longer internodes, larger leaves and decreased chlorophyll. These results are in accord with those reported by Stephan (520) who found that *Marchantia* grew abnormally in a light environment with a very high proportion of infra-red rays. However, Forster (132) reported no influence of infra-red rays upon the development of *Marchantia*. Apparently, normal growth of the tomato plant can be obtained under artificial light conditions where the infra-red is eliminated, provided the intensity of visible light is sufficient. It is possible that the difficulty experienced by Arthur (12) in growing tomato plants under long days with artificial light may have been due to the excessive proportion of infra-red.

As a result of the vast amount of accumulated knowledge concerning the radiant energy requirements for successful growth of economic crops, definite recommendations have been made recently for the practice of electric horticulture by Oden (368, 369), Roodenburg (444), Laurie (267) and others. For forcing cuttings and

seedlings in early stages of growth, about .003-.015 g. calories per  $\text{cm}^2$  per minute within the wave length limits of .4-1 micron is suggested by Oden (369) as being efficacious. The intensive cultivation of vegetables during winter required from .06-.12 g. calories per  $\text{cm}^2$  and the growth of sweet peas, beans, cucumbers, etc., need about 45 g. calories per  $\text{cm}^2$  day. Roodenburg (444) recommends, for supplementing daylight, the use of neon low voltage tubes which emit a red light of wave lengths absorbed by chlorophyll and therefore efficient in photosynthesis.

#### DIFFERENTIATION

Differentiation, in contrast with growth, may be viewed as a developing process whereby are obtained the forms and structures characteristic of different kinds of cells, tissues, organs and species. It seems clear that the morphological aspects of differentiation actually rest upon the different chemical stuffs and physical states within the cells of the organ or plant as it gradually passes from its beginning to its maturity. However, since the fate of a particular part of an individual may be determined by its relative position in the organism, or by chemical changes which may occur in some other totally different (but correlated) part, the organism as a whole must be considered. Differentiation may be regarded, therefore, as the sum of the physical and chemical changes which occur in the maturing organism and the diversified morphological expressions which arise as the result of these conditions.

Loomis (281) has discussed the "growth/differentiation balance" from the chemical point of view, namely, that growth depends on available water and the various elements needed for synthesis of protoplasm, while differentiation is conditioned mainly by carbohydrates. If growth is checked by some means (*e.g.*, by reduction in water or nutrient supply to the top) which does not impair photosynthesis, then the carbohydrates formerly used in protoplasmic synthesis accumulate and serve as raw materials for differentiation. Since the light régime is known to have profound influence upon physiological processes, such as photosynthesis, growth-substance formation and activity, pigmentation, etc., in growing plants, it should be expected also to have marked effects upon the production of their characteristic form and structure, *i.e.*, upon morphogenesis and histogenesis. Furthermore, it ap-

appears highly probable, in the light of modern investigations, that light exerts its influence in the development of plants in many ways which are yet imperfectly understood.

#### THE PLANT AXIS

An interesting aspect of the formative effect of light upon higher plants is that concerning the shoot/root ratio or the growth and development of the above-ground portion of the plant relative to that below the soil. Garner and Allard (146) found that distribution of food and intensity of growth in the shoot and root of many plants is governed to a considerable extent by the length of the daily exposure to light. Formation of bulbs, tubers and thickened roots, in those species where inheritance permitted, was enhanced by a length of day different from that favorable to vegetative increase. In soy bean, as the length of day was shortened below the optimum for stature or flowering, tuberization occurred. Potato showed vigorous vegetative shoot growth in an 18-hour day but a great proportionate increase in tuber formation occurred in a 10-hour day. The tropical yam, *Dioscorea alata*, under a 12-hour day yielded large tubers, but in a full summer length of day in high latitudes only small tubers were formed. Formation of bulbs was found to be increased as a result of a lengthened daily light period above the optimum for stem growth. For example, the onion produced good bulbs in long days and poor ones in a 10-hour day where the food was used for growth of the tops. Biloxi soy bean in short winter months failed to show top growth but the root system increased in the greenhouse. Maximov and others (308) observed that plants grown in shade have less extensive root systems and a greater per cent of dry weight. Simon (498) reported that shade or darkness delayed or prevented development of the plumule, stem and branches of *Bruguiera eriopetala* (Mangrove) seedlings but had no effect upon the root system. Dahlia cuttings grown in a short day yielded markedly thickened underground parts but in long day conditions there was but little storage of foods in the fibrous root system (598). Many tropical tuber-forming species of *Solanum*, *Ullucus tuberosus*, *Oxalis tuberosa* and *Tropaeolum tuberosum* were grown in different day lengths by Rasumov (424). Onions have been found to form bulbs in relatively long days (310). All species attained maximum vegetative de-

velopment and usually flowered abundantly in a long day, while the greatest production of tubers was afforded in short days. The greatest yield of tubers was nearly always produced by pre-treatment with a long day period followed by a short-day period. In several species, short days in the early history of the plants followed by long days in the latter period of development caused reversion of the tubers to stolons and an accompanying increase in vegetative shoots. Similar observations have been recorded by Tincker and Darbishire (543) and Hackbarth (178) for tuberous plants, and by Nightingale (359), Ulvin (553) and Eaton (110) for other plants. The ratio of tuber weight/top weight in potatoes has been found to range from 0.4 in continuous light to 2.0 in short days (106). Werner (583) found carbohydrate accumulation and good tuber formation when potatoes were grown under the following circumstances: when nitrogen assimilation was checked, at relatively low temperatures, under conditions of potassium deficiency, and in short days. Maximum tuberization occurred in medium days, at low temperatures and with nitrogen available. Furthermore, it has been found in general that plants which have grown under light conditions favorable to the accumulation of carbohydrate reserves are better able to resist unfavorable circumstances, such as drought and low temperatures (*cf.* 492).

Some investigators have not found differential growth of the shoot relative to the underground portions when plants were grown under varying photoperiods (*cf.* 572). Beets grown in various day lengths from 4 hours upward have been found to produce a maximum yield of leaves and petioles in 10 hours of light per day, with a decreased formation of leaves and an increased development of roots in longer daily exposures (248). Schick (470) observed that reduction in the day length to 12 hours had no morphological influence upon four potato varieties from Germany, but short days resulted in tuberization of three South American varieties, which indicates that photoperiod may have some effect upon the inheritance mechanism of plants which have been accustomed to the daily light régimes at different latitudes of the earth (*cf.* 106). It has been concluded by the majority of those who have found differential photoperiodic effects on axial growth that the accumulation of materials in fleshy underground parts is not due to an increased photosynthetic activity but arises from inability of the plants to use

the carbohydrates in excess of the current consumption for protein synthesis, respiration and cellular differentiation.

The effect of light intensity and duration upon the root/leaf mass ratio in *Raphanus* has been studied by Johansson (226). An increase in root growth was brought about by increasing intensities of light at all daily exposure periods ranging from 6 to 24 hours, but an increase in leaf mass was occasioned by increased light intensities only in the shorter exposures of 6 or 8 hours per diem. When the root weight as per cent of the total plant weight was plotted against day length, an increase was found up to 10 or 12 hours of light per diem, particularly at the higher intensities, but in longer days the relative root weights decreased. The weight of a unit area of leaf surface increased in bright sunlight and in longer days, provided the light was of sufficient intensity. The leaf weight of unit area was considerably increased in those plants grown in light which was filtered through glass. According to Probst (441), diffuse daylight falling upon the shoot portions of *Linum* and *Lepidium* retarded the growth of the shoot and stimulated the root. Direct illumination of the root retarded its growth. In *Sinapis* and other plants, prolonged illumination of the shoot followed by darkening resulted in increased root growth, possibly due to the mere increase in carbohydrates.

That the C/N ratio is closely connected with the proportional development of shoot and root has been demonstrated by many investigators (94, 199, 359, 433, 499) who have observed that relatively greater proportions of carbohydrates favored root formation. The inhibitive action of light in the formation of adventitious roots in *Tradescantia fluminensis* appears to be due to a mechanical hindrance in the hardened cortical tissues through which root initials can break more easily in the absence of light (319). According to Kellerman (234), in no case does the light period best adapted to food storage in bulbs, tubers, etc., coincide with the daylight period best adapted for upward or top growth of the particular plant under consideration. The nature of the regulatory action of light on the internal processes of the plant, other than those which merely determine the quantity of carbohydrate produced, await satisfactory explanation.

Whether the plant forms a leaf rosette or remains short, stocky and branched, or grows tall and slender, may be determined largely

by the quality and quantity of the incident light. A large group of plants is characterized by a pre-flowering period of growth which is limited chiefly to the formation of a leaf rosette, and when these plants are grown under conditions of a relatively short daily light exposure they remain in this stage for a long time without flowering. On the other hand, long days promote an upward shooting of the axis in this type of plant and flowering subsequently takes place. As an illustration, rosette plants of *Oenothera biennis*, when transplanted to a 10-hour day, showed basal branching but in full sunlight only a primary axis developed (146). Similarly, the form of radish plants may be controlled in a striking way by transferring the plants from one day length to another (cf. 553) or by varying the temperature (397). The axis in lettuce is known to remain short in short days, while the flowering axis elongates under long day treatment (448).

That elongation of the plant axis need not necessarily be followed by flowering has been shown by Klebs (240) who was able to induce terminal vegetative rosettes on elongated stems of *Semprevivum*. According to Daniel (91), *Allium porrum*, grown in low light intensity, flowered late and formed fasciations and proliferations in the inflorescences, and in some cases bulblets took the place of flowers.

Garner and Allard (146) have pointed out that as the light period becomes progressively less favorable for upward stem growth, the various responses of plants are those frequently associated with relatively dry conditions, *i. e.*, a tendency toward flowering, increased branching, pubescence, abscission and leaf fall, shortening of the stem, tuberization and increased underground development (cf. 337). Pobedimova (398) has also called attention to the effect of intense light on the formation of hairy stems in *Stellaria media*.

Chailakhian (72) grew winter wheat and barley in a series of different daily light durations, and found that various degrees of branching occurred, ranging from a flattened low type in plants exposed to a 10-hour day to a tall, compact habit under conditions of continuous light obtained with daylight supplemented by artificial illumination. The effect of day length upon the habit of cereal plants has been studied also by Hurd-Karrer (215), Purvis (223) and others. According to Hurd-Karrer (213), dormant rosette



stages in winter wheat are brought about by short days and this stage never appears with supplementary artificial light in the greenhouse. Also relatively high temperatures (20–23° C.) during the growth period can prevent rosette formation.

#### GROWTH REGIONS

Formative effects of the light period upon the growth habit in higher plants appears to be exerted through some modification in growth intensity of the various meristematic regions, which results in apical dominance, or branching, etc. It has been found that intense light tends to have a dwarfing influence, while weak light permits tall and spindly growth. Experiments with different wave lengths of light indicate that the shorter waves exert a strong regulatory action which gives plants their normal appearance, while the longer red waves act much like darkness, except, of course, that chlorophyll is developed and carbohydrates are manufactured. Descriptions of these photogenetic phenomena have been given earlier in the discussion.

The branched habit is indicative of a reduced apical dominance, and in the light of the recent contribution by Thimann and Skoog (536), dealing with the inhibitive rôle of auxin upon the growth of lateral buds, it would appear that some modification of hormone activity may be exercised by the light period. That the arrangement of foliar organs on the axis is also capable of modification has been indicated by the change from opposite to alternate phyllotaxy in hemp subjected first to a short day period followed by a long day régime (460). In these experiments, the photogenetic effect is exercised through some action upon the meristems.

According to Chroboczek (79), the growing points of *Beta vulgaris* which were destined to remain in a vegetative condition yielded very different values for the ratios of diameter to height as compared with plants which later developed seed stalks. Using various treatments to break the rest period in potato tubers, Denny (198) found that the very first chemical changes occurred in the region of the growing points.

Just what the nature of the correlative control over growing points in the plant may be, remains to be shown. It is known that the determinative influence of light upon an aerial part may be transmitted to a subterranean portion of the plant, and buds at the

nodes of stolons may be influenced to grow without a rest period and thus form short lateral shoots. When two coordinate branches of a *Cosmos* plant were subjected to different photoperiods, in all cases the vegetative branch, produced in the long day treatment, assumed a dominant influence over the basal portion which continued harmonious growth; the other flowering branch soon ceased growth and declined (146). Eghis (112) has reported that when soy beans were exposed to 10 hours of sunlight daily for 10 days early in life, flowering was hastened even under subsequent conditions of long daily exposures in later life, but only the first axis was capable of this "after effect." The lateral branches which arose later flowered at the same time as did the controls which had never been subjected to short days. Kondo and others (250) exposed the vertical halves of rice plants to short and long days, *i.e.*, one half to 24 hours and the other to 8 hours of light daily, and found that no part of the plant had any influence on the other parts.

However, experiments of Rasumov (425) have indicated the possibility for certain effects of localized photoperiodic stimulation to be readily transmitted morphologically downward but not upward. Our knowledge of the influence of auxins upon bud development and cambial activity (536, 511) lead to the assumption that some kind of organizing stuffs may be concerned in the differential expansion and maturation of the plant body.

#### TISSUE DIFFERENTIATION

That differentiation of tissues in the plant axis is conditioned by light to a considerable degree has been shown by investigations of etiolation and studies of the anatomy of plants grown under different light conditions. Bonnier (42), an early student of photomorphosis, observed the influence of continuous artificial light upon plant structure and anatomy. MacDougall (294) and Priestley (407, 414) have described the weak vascular and supporting tissues characteristic of etiolated plants, and the latter has shown how structural and morphological changes can be induced by even brief exposures to light. Various twining plants, when deprived of light for a few days, lose their power of circumnutation, due to some other cause than starvation (353, *cf.* 365).

Pfeiffer (390, 391) studied the anatomy of plants grown under different intensities and ranges of wave length. The full solar

spectrum rather than any part thereof yielded better developed tissues, as shown in the diameter and vascular anatomy of the stem, though it was difficult to conclude regarding the effects of radiation of different wave lengths, because the energy values were not equated. Deats (96) found that the amounts of phloem and xylem in stems of tomato and pepper varied directly with length of day. Variations in cell wall thickness and size of epidermal and cork cells varied in a similar manner with the light duration. Reid (437) investigated the effects of light on development of seedlings and reported that carbohydrate synthesis promoted increased cell wall thickening in the xylem and more extensive lignification of the phloem fibers. Penfound (386) observed that the anatomy of *Helianthus* and *Polygonum* grown in full sunlight differed from shade grown plants in that the roots and hypocotyls possessed a greater diameter and greater area of xylem, and more and thicker walled mechanical elements. In the castor bean, the diameter and amount of xylem in the roots, the xylem area and size of the cells and wall thickness in the stem varied directly with the insolation and soil moisture content. Doroshenko (104) reported that flax exposed to a limited day length grew less in stem thickness and showed decreased development of xylem and phloem and increased diameter of the pith.

#### LEAVES

The form and anatomy of leaves in some plants is affected by light. Bright light produces relatively thick well-differentiated leaves, while leaves growing under reduced illumination are thin and poorly differentiated. According to Lundegardh (291), many plants, such as beech and geranium, have the ability to form typical "shade leaves" in the shade and typical "sun leaves" in the sun, while other plants form only "sun leaves" or only "shade leaves."

The effect of light intensity upon the number and size of the chloroplasts present in leaves has been studied by Lubimenko (cf. 307, 411) and by Euler and others (121). Typical sun plants possess smaller chloroplasts than shade plants. Adaptation to shade is shown by reduction in leaf thickness followed by decrease in the number of plastids together with an increase in their individual dimensions and chlorophyll content (411). Geiger (155) described some of the peculiar properties of an extreme shade plant,

*Aspidistra elatior*, which thrives in habitats furnishing 6-8 per cent of normal sunlight intensity and is capable of surviving without etiolation in 1/200 normal sunlight. The leaves possess 1/10 as many stomata as many sun plants and the intercellular volume is about 19 per cent of that of sun plants. Reed (432) found the number of stomata per mm<sup>2</sup> in the shaded leaves of *Citrus limonia* (Eureka lemon) reduced to about half the number in the sun leaves of the same tree. Haberlandt (177) has given much attention to the problem of sun and shade leaves in the *Crataegomespili*. *Cra-*

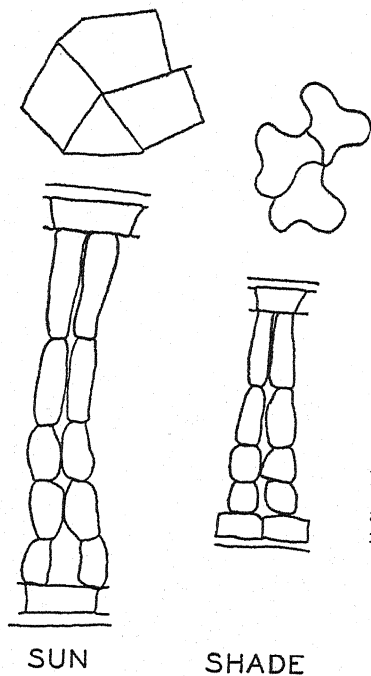


FIGURE f. Variation in the structure of *Crataegus monogyna* leaves grown in direct sunlight and in shade. "Sun" leaves are thicker and possess more palisade tissue than "shade" leaves. Also the side walls of the epidermal cells are straight in leaves which were grown in bright sunshine and curved in leaves which developed in subdued light. After Haberlandt (177).

*taegus monogyna* is characterized by having straight walled upper epidermal cells and thick leaves with three palisade layers in the sun, and curved epidermal cell walls and thin, less differentiated leaves in the shade. *Mespilus germanica* possesses curved walls in the upper epidermal cells of both sun and shade leaves, but the shade leaves are decidedly thinner and less differentiated. Experiments with *Helianthus* (386) grown in full sunlight and in the shade indicate that with an ample water supply, sun grown plants

possess larger leaves with larger epidermal cells, larger mesophyll and midvein, and more numerous stomata. As Blackman (35) has shown in tomato, the increase in leaf area per unit time may decrease as the light increases, though the dry weight continues to gain. This again suggests that growth regulation is influenced by light through processes other than the synthesis of food materials.

The formative effects of light upon leaves have been described by Priestley (407) and by Ewing and Priestley (413) as a result of their work with the phenomena of etiolation. The outstanding thing about plants grown in absolute darkness is the failure to develop leaf laminae of any appreciable size. Trumpf (550) was able to demonstrate the action of short light exposures in bringing about leaf expansion. Brief exposures to blue or white light when the plants were under chloroform anaesthesia, or at low temperatures ( $3-4^{\circ}\text{C.}$ ), caused some kind of action which was carried over in the plants so that later under ordinary atmospheric and temperature conditions normal development took place in the dark. Priestley believed that photochemical action on the inert materials in the cells must have taken place under these conditions, and that this unknown substance exerted its influence upon future development.

Li (276) has observed that both formative and extension phases of leaf development in *Ginkgo* require light. For formation of leaf primordia, the light requirement is low, even 2 m. c. sufficing for production of foliar initials. Stronger light is required for the extension phase of leaf growth, and different parts of the blade have different light requirements for expansion. Reid (436) reported that seedlings grown in light in the absence of  $\text{CO}_2$  had larger leaves, and cotyledons which weighed more than those of seedlings grown in darkness.

The photogenetic effectiveness of different regions of the spectrum upon form has been studied at length by Teodoresco (533) who observed that blue light hinders petiole elongation and favors growth of the leaf surface. Bizarre filamental forms were noted in liverwort and moss gametophytes grown in red light and darkness but blue or white light induced normality of form. Funke (138) has discussed the results of extensive experiments with *Potamogeton natans*, *Alisma*, *Plantago*, *Sagittaria sagittifolia* and *Sagittaria subulata* which form two sorts of leaves, namely, linear and immersed in their youth, and later, aerial and well differentiated

blades. In red and green light the leaves never develop beyond the linear stage, in blue light the aerial leaves with blades are formed just as they appear in ordinary daylight. When growing plants were exchanged from red to blue, and *vice versa*, an immediate adaptation to the new radiation environment took place. That intensity of light along with the nutrient salt ratio may have some effect upon leaf form in *Potamogeton perfoliatus* has been suggested by Pearsall (384). Popp (401) and Pfeiffer (391) both reported that leaf development was abnormal when growing plants were deprived of the blue end of the solar spectrum. The growth pattern of *Tropaeolum* leaves in normal and reduced illumination has been analyzed mathematically by Smirnov and Zhelochovtsev (502). Variation in the light conditions modified the fundamental field of growth; and ordinary daylight, as compared with shade, stimulated leaf expansion in the early stages of development from the bud. The recent correlation of growth-substance concentration with growth vigor in *Nicotiana* leaves (17) appears to be of considerable significance for the explanation of differential growth patterns which are subject to modifications by the changing environment.

From the standpoint of light duration in relation to leaf development, it has been found that the growth period is longer and the size greater in leaves subjected to relatively long light periods alternating with short periods of darkness than when grown in continuous light (430). Maximov (305) grew plants under artificial light and reported that differentiation of mesophyll and palisade tissues was greater in continuous light than in alternating light and darkness. Ulvin (553) found that radish leaves were thinner in plants grown under continuous light, as compared with those grown in alternating light and darkness. Deats (96) reported the greatest leaf thickness and size in tomato and pepper when grown in relatively long days. However, Pfeiffer (390) observed that thinner leaves were produced in pepper and thicker leaves in tomato grown in long days. These differences of leaf structure observed in various species in response to light conditions may be regarded as the result of the interaction of a number of factors such as the genetic constitution of the plants, the intensity, quality and duration of radiation, and the conditions imposed by soil nutrients and water supply.

The phenomena of abscission and leaf-fall in relation to photoperiod has been investigated by Garner and Allard (146) in *Rhus glabra*, *Liriodendron tulipifera* and other plants. Several individuals of these species were grown outdoors during the summer and were transferred into the glass house in early autumn, where one lot of plants was given the light of natural day length and the other was given daylight supplemented with artificial light of low intensity in the evening. The lot of *Rhus* plants which were provided only with ordinary daylight dropped their leaves, but the other plants in long-light periods retained their dark green leaves. The *Liriodendron* trees treated with supplementary light also kept their leaves and grew new ones, while the other lot of trees abscised their leaves and remained dormant. This type of behavior was not characteristic of some other species of plants in the experiments. Kind (236) has observed that in autumn, the leaves of poplar, linden, chestnut, etc., situated near street lights remained green on the trees from two to three weeks longer than usual. Kramer (252) has found that yellow poplar seedlings given supplementary electric light until midnight never became dormant but continued to grow through the winter. These observations appear to be of importance in view of the newer knowledge concerning plant growth-substances which prevent abscission of petioles (*cf.* 581).

#### REPRODUCTION

The formative influence of the light period upon plants is of great interest in connection with initiation and suppression of sexual reproduction. In 1920 Garner and Allard (145, *cf.* 144) announced that the behavior of plants with respect to development of reproductive stages when grown under different daily light exposures would permit their classification into three groups: (a) the indeterminate type which is not sensitive to the photoperiod over a wide range; (b) the short-day type in which flowering is promoted by relatively short days; and (c) the long-day type in which reproductive processes are favored by long daily periods of exposure to light. The critical light period which may be termed the dividing line between long and short-day plants appears to lie somewhere near 14 hours of light per diem, but in testing the photoperiod type of any plant it is usually a safe procedure to grow the unknown under a decidedly short period of 10 hours and also

under a markedly long period of 18 hours (144). If flowering be materially delayed under the short-day treatment, the plant should be regarded as belonging to the long-day type as, for example, *Avena*, *Triticum*, *Raphanus*, *Rudbeckia*, etc. If the short-day treatment hastens flowering, the plant should be characterized as a short-day type as, for example, *Aster*, *Cosmos*, *Nicotiana*, *Salvia*, etc. In case the time from planting to flowering be substantially the same under the two treatments, the plant may be considered as belonging to the indeterminate group, as in the case of *Fagopyrum*. Since the discovery of photoperiodism, scores of papers have been published on the photoperiodic response of numerous species of plants, including weeds, flowers, cereals and vegetables. According to Moschkov (337), many trees and shrubs also exhibit photoperiodic response. Among woody species, *Poinsettia* and *Bougainvillea* are short-day plants, and *Hibiscus syriacus* is a long-day type (3). The photoperiodic characteristics of numerous kinds of plants have been recorded in tables compiled by Redington (429), Czaja (90) and others. An extended discussion of the detailed data is not possible here.

Garner and Allard (149) found that darkening plants in the middle of the day for periods of 1 to 5 hours generally failed to influence the reproductive activities in any way comparable to the effects obtained by excluding early morning or late afternoon light. The effects of midday darkening were essentially those obtained in long days. Under controlled conditions, series of different plants, including *Cosmos*, *Delphinium*, *Rudbeckia*, etc., were exposed to different short alternations of artificial light and darkness ranging from 6 hours to 5 seconds. As a rule, all the alternations were favorable for flowering in the long-day plants, and unfavorable for flowering in the short-day plants. The plants behaved as if they were exposed to a long day or to continuous illumination in so far as flowering was concerned, but very important differential effects were obtained with respect to vegetative growth, as discussed previously. The differences obtained in growth and reproductive responses under the well-controlled conditions of these experiments suggest that of the many reactions which are set up in the plants, certain processes must be affected more than others by the light régime, and in some instances the balance swings in favor of vegetation while in others the final trend is in favor of reproduction.



The significance of the photoperiod in growth and maturation of plants in different regions of the earth has been studied by many workers (286, 309, 427, 542, 543, 310, 594, 122, 470). The results may be summarized as indicating that with increasing latitude, light and temperature conditions become less favorable for flowering in short-day plants, while long-day plants, having a high critical light period, are unable to flower and fruit successfully except at high latitudes. It is believed (145) that annual, biennial and perennial plants are largely expressions of the effect of the prevailing seasonal range in day length, and that the photoperiod is an important factor in the natural distribution of plants (286). Doroshenko and Rasumov (105) reported that many varieties of wheat, barley and beans which have been grown for a long time at higher or lower latitudes possess the characteristics of short- and long-day plants, respectively. It may be that environment (*i.e.*, daily photoperiod) has modified the flowering expression of different races of plants at different latitudes and that these characters have become more or less fixed. Little is known concerning the genetics of the time required for flowering, though Rasmusson (422) has found two main factors in *Pisum* showing partial dominance toward lateness.

Laboratory determinations of the influence of wave lengths, optimum intensity and duration of the light exposure upon plant development have been possible only as a result of the invention of efficient and appropriate mechanical and electrical apparatus in recent years. The experimental technique in light investigations has involved the use of elaborate equipment in many instances where it has seemed desirable to control the temperature and moisture, as well as light conditions (148, 12, 268, 400, 490). Hendricks and Harvey (194) used specially constructed apparatus which permitted growth to maturity under artificial light as the sole source of energy. The range of intensity employed in the different series of experiments was from 50 to 10,632 f. c. For each of the 45 kinds of plants there was reported a certain intensity range, narrow or broad in different instances, in which blooming and seed production took place. Flowering occurred for different plants under continuous illumination in different ranges of intensity (with the temperature maintained between 20–25° C.) as follows: maize 500–3,000 f. c., tobacco 800–10,000 f. c., squash

500–750 f. c. Maximov (305) found that wheat and peas bloomed in continuous light, but flowering was delayed in buckwheat and prevented in soy bean under the conditions of the experiments. Substantial increases in the yield of sweet pea flowers have been obtained with electric light supplementing daylight (190). Adams (2) was able to grow the castor bean from seed to seed under artificial light, and many investigators have obtained increased yields of fruit and several generations of cereal grains in one year by employing artificial light supplementary to daylight (187). McKinney and Sando (313) obtained mature seed from winter wheat in 100 days by growing plants in an 8-hour day at 50–60° F. during the first 54 days followed by 17½–18½-hour days at about 70° F.

Long ago it was found that flowering plants were not able to form functionally mature stamens and pistils (294) in complete darkness. Recently, Colla (83) has reported that hyacinths with ample food reserve, when exposed to ultra-violet radiation, flowered and shed pollen, while control plants kept in complete darkness failed to flower. Wann (568) observed that fruiting in *Marchantia* was promoted by the use of supplementary weak electric light and Schappelle (465) found that the orange-red region was effective in this respect. Garner and Allard (147) showed that the flowering conditions which were produced in illuminated portions of a plant were able to bring about the flowering effect in other darkened branches of the same plant. The amount of light energy which is required for normal development and formation of functional flowers depends upon the genotype and, furthermore, the effectiveness of light in the formative processes appears to vary considerably during the ontogenetic history of the individual plant (71, 369). Ramaley (417, 418) has reported concerning the influence of ordinary electric light supplemental to daylight upon the flowering of 100 different species of greenhouse plants. The researches of Rasumov (426), Withrow (591), Wenger (576) and others lend support to the earlier findings of Garner and Allard (146) that weak supplementary illumination of approximately one thousandth the intensity of sunlight is effective in promoting flowering response in long-day plants.

Though sunlight is probably a better source of energy for growing plants than artificial sources now available, the use of tung-

sten incandescent lamps and neon discharge tubes has been worked out experimentally with great success in many laboratories (369, 444). Oden (369) and associates have demonstrated the value of supplementary lighting to force seedlings and to enrich the flowering of certain plants. The fundamental principles of electrohorticulture and their applications to the culture of commercial greenhouse crops have been discussed by Greene and others (171) and by Laurie and Chadwick (267). The observations of Oden, with respect to the proportions of different wave length components and the effects upon plant development, are of considerable interest. In *Convallaria*, the proportionate increase in the red light emitted by tungsten lamps operated at reduced voltage favored flowering, while light richer in shorter wave lengths, obtained by operation at a higher voltage, retarded flower development. In forcing, it was claimed that the infra-red spectrum ranges are necessary for normal elongation of the flower stock. Development was most favorably promoted in long-wave radiation in pea, raspberry and string bean, while growth was enriched by short-wave rays in radish, dill and lettuce.

The significance of the quality of light in photoperiodical response has been further elucidated by the extensive experiments of Rasumov (426) using long-day wheat, oats, bean, poppy, *Cicer*, pea and hemp, and short-day millet, maize, soy bean, hemp, potato and Mexican bean. These plants were grown on 10 hours and 17-18 hours of daylight alternating with darkness, and with 10 hours of daylight supplemented by filtered light of six different wave length ranges and controlled intensities. By replacing the dark hours with different wave length bands of equated radiant energy, it was found that the long wave red rays were active in promoting processes of reproduction in both the long- and short-day plants. The shorter wave lengths of the green, blue and violet, when applied separately or together, acted like darkness with respect to initiation of the flowering stage. There was some variation in the quality and intensity of light required by different kinds of plants, but for all the crops investigated, the minimum intensity was very low, *e.g.*, of the order of 10 lux. Since the magnitude of the radiant energy capable of determining the course of differentiation was well below the compensation point, it was assumed that the formative action of light is not exerted indirectly through

photosynthesis of carbohydrates, but directly through some mechanism which regulates the use which the plant makes of its food.

Additional evidence in support of the idea that very low intensities of the longer rays of light are effective in promoting the flowering response has been given by experiments performed by Wenger (576). Aster, a long-day plant, was grown in daylight supplemented with tungsten light over a series of varying intensity for 10 hours each night. Maximum and earliest flowering occurred in all instances where the plants received supplementary illumination of the lowest intensity, *i.e.*, .3 f.c. With increasing intensity, flowering was delayed and reduced in amount, and vegetative growth was increased. Withrow (591) reported experimental results obtained on the flowering responses of pansy, aster and stock grown in the winter daylight supplemented with artificial radiation of controlled intensity and selected wave lengths to maintain an 18-hour day. With supplemental white light even as low as 1 f. c. marked acceleration of flowering production was noted. In the experiments with supplementary blue, green, yellow, orange-red, red, extreme red and infra-red radiation, the most marked responses were produced by radiation in the region 620 to 720 micro  $\mu$ .

The response of plants to relative length of day may be influenced by temperature and humidity conditions obtaining during the period of growth. Garner and Allard (146) found that lower temperatures tended to conserve the stored energy and delay the time of flowering due, probably, to regulation of the respiratory activity. Gilbert (158) observed in soy beans and cotton that there was retardation of flowering at lower temperatures and higher humidity, while flowering of *Cosmos* was speeded by these same conditions. Formation of flower primordia in *Xanthium* was considerably influenced by temperature, but *Salvia* and buckwheat exhibited no particular response to varied temperature and humidity conditions in the range employed. Adams (2), Bolas (40), Blackman (35) and others have emphasized that temperature must always be taken into consideration in experiments dealing with the rôle of light. Furthermore, it has been demonstrated that the temperature factor may control the vegetation/reproduction balance in a very striking manner in many plants, such as celery, cabbage, beet, etc. (539, 79, 323).

The interrelationship between temperature and photoperiodic stimulus has received considerable attention in Russia where the principles of photoperiodism and jarovization have been found to be of great practical importance in the successful production of economic crops. In 1918 Gassner (151) reported that the length of time required until flower formation in winter wheat decreased with decreasing temperature at germination in the range plus 26° to minus 1° C. In recent years, the conversion of winter plants into spring plants has been accomplished on a large scale either by pre-treatment of germinating seeds in a definite manner (292, 306) or through regulation of daily light exposure during the growth period. Jarovized seeds differ from ordinary unjarovized seeds in that, other factors being equal, the former develop into ordinary spring plants and mature within one season. Chialakhian (72) exposed barley, wheat, etc. to different light régimes and found that long days favored the maturation of grain in the fall of the same season (*cf.* 313). Purvis (416) reported that the germination temperature, as well as the photoperiod, influences the growth and maturation of winter rye. Winter rye, germinated at 1° C., behaved as a long-day plant, but when germinated at 18° C. it responded like a short-day plant in that differentiation of flower initials proceeded more rapidly in short days. If differentiation of flower primordia is accepted as the basis of classification of photoperiodic types, then the spring cereals are true long-day plants. The winter varieties germinated at high temperatures are short-day plants with respect to flower initiation but demand long days for subsequent maturation, while the winter varieties germinated at low temperatures are similar to spring varieties, *i.e.*, they behave as long-day plants. Gilbert (157) grew cocklebur plants in long and short days and under conditions of high and low temperature for each photoperiod. When subjected to high temperatures, flowering occurred in the short day treatment in two weeks and under long day conditions in seven weeks, but in a low temperature environment the vegetative period was prolonged in short days to 16 weeks and in long days to 13 weeks.

#### SEX REVERSAL AND REJUVENATION

Variations in light appear to be capable of bringing about a shift in the physiological trend of some plants to the extent of

causing such marked phenomena as rejuvenescence, sex reversal, etc. Vegetative growth of a plant frequently terminates with formation of fruits which are believed to divert the available nitrogen away from the vegetative growing points (345). Sande-Bakhuyzen (454) has shown that the water content of vegetative organs of wheat decreases rapidly at the time of fruit formation, and exhaustion and death of all parts except the fruits finally take place. Murneek (343) considered gametic union to be the cause of localized metabolic gradients which exert a detrimental influence upon the other metabolically weaker parts of the plant. In whatever manner senescence is hastened through the accomplishment of reproduction, it seems clear that light periods which are not favorable for flower production ought to defer the arrival of old age. The youthful activity of the vegetative meristems can be prolonged by appropriate light periods (146) and by relatively high temperature conditions (79). Klebs (240) was able to control vegetation and reproduction by manipulating the light and inorganic nutrients. Garner and Allard (146) reported rejuvenated vegetative activity after flowering by manipulating the photoperiod in appropriate ways for soy bean, wild aster, *Poinsettia* and other plants. Schaffner (460) reported three such rejuvenations in individual hemp plants wherein flowering was induced by short days alternating with a period of vegetative development under artificially controlled long day conditions.

Since control of vegetation and flowering can be exercised by light, it is not surprising that sexual expression fluctuates with variations in the photoperiod. Photoperiodic modification of the processes of vegetation and reproduction in maize is well known (112, 117). Eghis (112) grew a race of maize under 12 hours of daily light and obtained complete reversal of the staminate flowers to the functional carpellate condition, so that ears developed in place of tassels. Also, Schaffner (461, 462) has reported that the male flowers in maize may be suppressed completely by decreasing the light period to a suitable day length. Furthermore, the same author (463) has shown that staminate hemp may undergo sex-reversal and develop carpellate flowers when grown in the short winter days of a north temperate latitude. McPhee (314) has found that the time of flowering of hemp is controlled largely by the relative length of day and night, a seven

hour period of daylight accelerating flower formation to an optimum extent. Furthermore, more intersex types were formed in the winter months. Tiedjens (540) reported an increase in the number of staminate flowers in cucumber grown in abundant light, and an increase in pistillate flowers under reduced light conditions. Gardner (143) found that low carbohydrate supply in strawberry at the time of flower bud differentiation led to a failure of female flowers in a variety which is normally hermaphroditic. If sexual expression may be assumed to result from differential rates of activation of the genetic factors for male- and femaleness (*cf.* 28, 84, 165), then it is no more difficult to account for variations in sex than to explain the shifts in the growth/differentiation balance or any other phenomenon intimately associated with the course of metabolism.

The relation of light to fruit development has been shown to be of great importance through the hastening or, in other instances, through the repressing effect upon flower formation. That a more direct relation may exist has been indicated by Gray (170) who found that, though a moderate reduction in intensity was without effect upon the set of fruit in sour cherry, heavy shading with burlap caused abortion of the embryos. In order to explain the behavior of the fertilized ovary as a growth center, the suggestion has been made that hormones may be concerned in the establishment of a metabolic gradient (*cf.* 311, 345). Too few facts are known as yet to permit the formulation of a sound physiological explanation for the morphologically well known story of reproduction.

#### DIFFERENTIATION OF THE PLANT AS A WHOLE

Recent studies of the differentiation of growing organisms have led to the suggestion that the morphological plan of the individual is determined by a basic chemical ground plan of growth which is capable of modification within wide limits in space-time (350). According to the scheme of Huxley (219), the processes involved in growth and differentiation in plants may be classified into three phases: (1) chemo-differentiation, which includes invisible determination of the specific fates of different regions of the embryo or generating part through orientation of the chemical pattern; (2) histo-differentiation, which embraces tissue differentiation,

and the assumption of the definitive general form-plan; (3) auxano-differentiation, which consists of the subsequent quantitative growth changes.

The action of light as a stimulus causing living plant cells "to adopt appropriate rôles from a limited repertoire" (*cf.* 537) according to the genotype, has been discussed already at considerable length. That photochemical reactions take place in plants is well known from extensive investigations concerning chlorophyll and photosynthesis of carbohydrates (283, 119, 570, 19), the formation of anthocyanin pigments (9, 370), the movements of stomata (459) and the effect of light upon the synthesis of plant growth-hormones (17, 347, 536). Further indirect evidence comes from the great volume of literature dealing with phototropic response to specific wave lengths of radiation (*cf.* 65), the formative influence of brief light exposures upon etiolated plants (407, 437, 550) and the collective data concerning the development of plants grown under controlled intensity, wave length and duration of radiation (12, 146, 194, 426, etc). Also certain physical effects of light are known in the modification of normal polarity by photo-electric potential (47, 162, 489, 565, 566), and in the regulation of water requirements through transpiration (13); also in other ways, as suggested by light growth reactions (65, 70) whose specific mechanisms have not yet been fully explained. On the assumption that metabolic and structural differences between species are causally related to specific differences in chemical constitution, the question naturally arises as to how the various morphological characters of form, size and structure are attained in a correlated fashion. How does light determine the final swing of the balance of processes in a given direction which results in the successive initiation of vegetative organs, the formation of flower initials, the determination of sex, or the rejuvenescence of the aging individual? The answer does not yet appear simple. Histo-differentiation is obviously at work in the first formation of the organ-rudiments destined to become root, branch, leaf, flower or fruit. It has been stated that, in general, the younger the tissue, the lower the C/N ratio (200), but regulation of the proportionate amount of carbohydrates in the plant through light action and by other means has not been found capable of explaining organ differentiation. The available evidence from many dif-



ferent sources suggests that light influences the chemical (146, 199, 244, 536), and physical (79) conditions which are of the utmost significance for the initiation of organ primordia as well as for the maturation of the differentiating tissues, but the exact nature of the processes which accomplish these results is not known.

After organs have been initiated by the interaction between genetic factors and environmental stimuli, or in whatever specific manner, their subsequent development takes place in an orderly fashion according to the well known laws of differential growth. Huxley (219) has shown clearly by numerous examples in animals that over a considerable range of auxano-differentiation, the relative growth rate of the organ to the relative growth rate of the body remains constant. That the constant differential growth ratio between different parts of a plant may be influenced by light has been demonstrated (219, 383). For example, the differential growth of shoot weight compared with root weight in normal and etiolated seedlings of *Pisum sativum* yielded values for the differential growth ratio ( $k$ ) ranging from .90 to 1.15 in the light and 1.75 to 2.65 in the dark. This simply means that the shoot grew more rapidly than the root in the etiolated plants, while the shoot and root grew at approximately the same rate in the normal plants. It has been shown in many instances that growth vigor in different organs of a plant can be modified greatly by variation of the photoperiod (135, 508) and that with proper adjustment of the day length the rate of vertical elongation and final stature may be increased or decreased over a very great range.

Certain aspects of the light influence in morphogenesis may be explained in part, on the basis of specific substances (hormones) and their distribution due to normally occurring electric potentials (162, 246, 419, 565, 579). As an example, there may be mentioned the production of plant growth-hormones in buds, leaves, (17, 510) etc., and their distribution in a polar manner to other portions of the plant body where effects are brought to bear on the processes of growth and development. In other instances, the effect of light upon the organization of the plant as a whole may be carried out through a somewhat different mechanism. Harper (186) observed that in the myxomycete *Polysphondylium*, normal alternation of day and night favored the production of more and

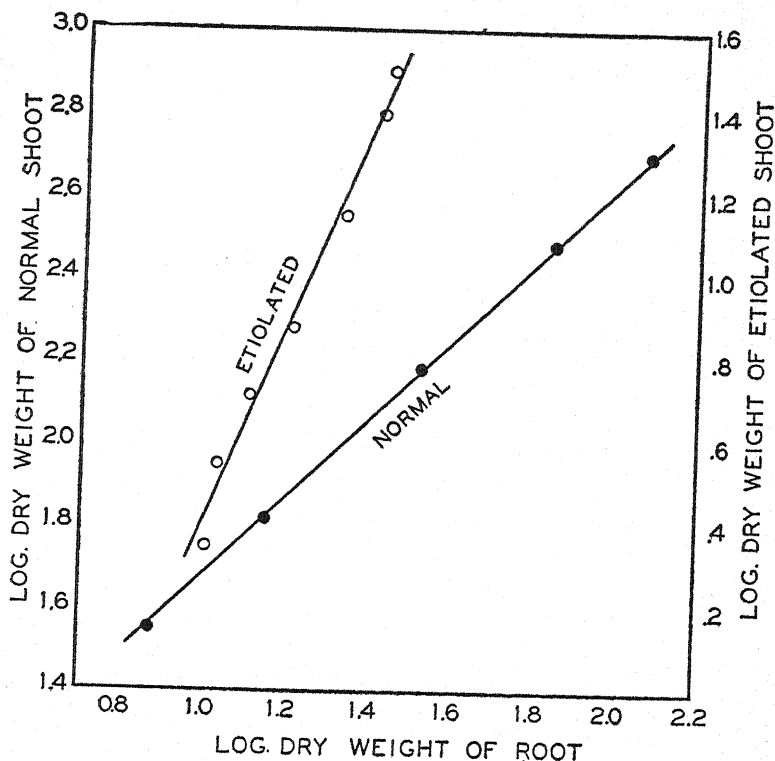


FIGURE g. Constant differential increments in the dry weight of the shoot and the root of the garden pea (*Pisum sativum*). The young plants were germinated and grown in nutrient solution. The etiolated plants were kept in darkness, and the normal plants were exposed to ordinary daylight and darkness. Note how the dry weight of the shoot in the etiolated plants increased with relatively great rapidity. Data from Pearsall (383).

smaller plants, while almost continuous darkness brought about the production of larger and fewer plants. These observations were interpreted as "indicating that cell growth and division go on equally well under both sets of conditions, but that the processes of aggregation and integration tend probably in darkness to favor the gathering of the myxamoebae from larger areas to the points of integration, thus resulting in the formation of fewer and larger plants per unit culture." It appears that modification and development of the form of cells, organs and organisms may or may not occur simultaneously with growth. From this viewpoint, the cells of a multicellular organism are considered as behaving more or

less independently in growth and differentiation and their integration into characteristic multicellular tissues, organs and organisms is a matter of their physical and chemical interrelationships.

It has been shown how light affects the physical and chemical constitution of plants. The light synthesis of food materials and "organizing" substances, and some degree of light control over their activity in the processes of integration and correlation of the whole organism has an important significance for morphogenesis. It is relatively easy to understand how light can bring about a quantitative effect upon such characters as gross size and form, and even upon microscopic shape and structure of cells which comprise the tissues of the plant, when the organism as a whole is structurally considered as the sum total of all its parts. Size and form in the plant depends upon the number, size and form of the constituent cells, and the characteristics of these individual cells depend upon the nature of the molecular pattern of which they are comprised. This is almost as far as it has been possible to carry the story, but it is not quite the end. It has been shown, further, that the quanta of light rays which are absorbed by the atoms of matter become activated so that photochemical reactions ensue, changes in temperature occur and photoelectric potentials are developed. Photomorphosis (the configuration brought about by light) in plants would appear to rest upon both qualitative and quantitative aspects of the physico-chemical situation. The synthesis of special compounds in photo-chemical reactions, the differential catalysis of physiological processes, and the correlation of the whole plant by electrical potential and other interchanges, such as may be conditioned by surface tension, protoplasmic streaming, etc., suggest a variety of possible ways by which light may contribute to growth and differentiation of the plant body. As Dixon (102) has written, "a long chain of energy and of material transformations, imperfectly ascertained and understood, usually intervenes in biological phenomena between what we recognize as the cause and the result." From the line of reasoning developed in this review of the literature, it may be concluded that the relation of light to the structure and pattern of plants depends upon its quantum action in the minute electrical structure and pattern of matter. The many gaps in the record, due to the complexity of the material and of the processes, remain for future investigators to complete.

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# THE BOTANICAL REVIEW

VOL. II

APRIL, 1936

No. 4

## THE ESSENTIAL NATURE OF CERTAIN MINOR ELEMENTS FOR PLANT NUTRITION

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Until about the beginning of this century, ten or eleven elements only were generally considered as essential for normal growth of plants. The presence of other elements in minute quantities was recognized in many plants, but their possible association with nutrition and growth was not understood. The activities of the French investigators from 1897 onwards focussed attention on the function of these minimal traces of elements, and the work of Bertrand (9) on manganese, Javillier (70) on zinc and Agulhon (1) on boron may be regarded as the foundation of the widespread investigations which are now of such practical economic importance.

The French school claimed that some of these minor elements were essential for the full development of certain plants, but the experimental difficulties in obtaining adequate proof were not at that time fully overcome, and for a time the matter remained of academic, rather than practical, importance. Still, interest had been awakened, and much experimental work resulted in many and varied claims being made for different elements—claims which frequently were not borne out by other tests under different conditions. In 1923, however, Warington (160) was able to prove conclusively that a trace of boron is absolutely essential for the development of *Vicia faba*, as in its absence the meristematic tissues die and growth is impossible. Since then, investigations all over the world indicate that boron is probably essential for all plants in varying degree and that certain obscure plant diseases may be due to a deficiency of this element.

Although it is more difficult to get clear-cut proof of the essential nature of manganese for all plants, the whole body of evidence

is now so strong that this statement is generally accepted as fact. The small amount that is necessary and the closeness of the association of manganese with iron render this element peculiarly difficult for experimental treatment. Copper and zinc have also attracted much attention and their value in certain cases seems evident, though they cannot lay claim to the importance of manganese and boron.

Work has also been done with a wide range of other elements, often with conflicting results and in no case affording proof of their universal value. Stimulation of growth is frequently recorded, but up to the present most of the information gained deals with the relative toxicity of larger amounts of the elements. It must not be forgotten, however, that there is a possibility that certain elements may prove to be essential for certain plants, though not for all. If this is the case, the proof of the association of the particular element and plant will be difficult to obtain by deliberate investigation, but is more likely to be the result of a fortunate chance observation.

During the last thirty-five years the literature on the relation of minor elements to plants has become very extensive. Willis' (165) bibliography covers between 2000 and 3000 references with abstracts, and Jacks and Scherbatoff (69) give more than 350 others, but even so, the whole field is not covered. Much of this literature deals with the toxic and fungicidal aspects of the subject, but in the present review attention is chiefly confined to work done on the possible essential nature of certain elements for plant growth during the last five years. In spite of this narrowing of the subject, it is almost invidious to select certain papers for comment and to omit others, and such omission in no way implies that any particular piece of work is of less value than those actually quoted. Anyone who is interested in any particular element is strongly recommended to refer to the two bibliographies above mentioned in order to get a more adequate idea of the available information.

#### BORON

The essential nature of boron for many species of plants was fully established before 1930, and the possible connection of boron deficiency with certain plant diseases was already under consideration, as in the extensive investigations of Mes on "topsiekte" in

tobacco (102). During the last five years the practical aspect of the matter has been widely investigated, and boron deficiency is now suspected to be the cause of a variety of obscure physiological diseases which cannot be traced to insect or fungus attack.

Heart-rot, crown-rot, or dry-rot of sugar beet is characterized by the blackening and death of the central leaves, coupled with discoloration or rotting of the upper part of the root. Late in the season secondary growing points develop numerous small leaves, giving the plants a very dense, short, green top. Where the disease is prevalent, the yield is often very low and the sugar content considerably decreased (137). It has been found repeatedly that the application of small quantities of boric acid or borax to the soil effectively prevents or cures the trouble (18, 21, 49, 63, 74, 141). The necessary quantities range from about  $4\frac{1}{2}$  to 9 pounds of boric acid, or 10 to 20 pounds of borax per acre, the best time of application being at or before sowing (16, 17, 43, 106). Where the disease occurs on mildly acid soils it is associated with lack of boron compounds, but it is also found on alkaline soils which contain as much boron as should suffice for normal growth. In the latter case, it seems probable that the boron is in some way locked up and rendered unavailable for the plants. This hypothesis is strengthened by the increasing amount of heart-rot that is being found in districts where the soil is limed, pointing to a gradual withdrawal of the boron from its available condition. In England and Scotland the disease is only gradually being recognized and reported, but an increase of the trouble is anticipated if heavy liming of the sugar beet areas is carried out, as the danger of excessive calcium is already realized (13). The association of heart-rot with boron deficiency has been confirmed in controlled water culture experiments, in which similar symptoms can be induced. It is also claimed (8) that the presence of boron increases the resistance of sugar beet to poisoning by the heavy metals. Solunsky (153) has examined the growth conditions which determine the effect of boron deficiency on sugar beet and suggests that water relations are of great importance. His conclusions are that the increase of moisture in the soil during the first half of the vegetative period stimulates the development of foliage and aggravates the disease, while at the very end of the vegetative period, in some cases, it may result in recovery of the plant. Fron (44) also suggests that



one of the chief factors causing heart-rot is lack of water caused by the drying out of the soil.

Brown-heart of turnips is a parallel disease which has been successfully controlled in Canada (89, 90) by the application of ten pounds per acre of borax. This has since been confirmed by experiments in Scotland and Wales (114a, 169).

Boron is now generally recognized as essential for tobacco, deficiency producing characteristic symptoms of disease (152). As generally happens, the meristematic tissues are primarily affected, the stem apices die and flowering is inhibited, the effect on roots and leaves being less marked. Diseased leaves are richer in starch and sugar than healthy ones, possibly because the disorganized phloem interferes with normal transport (146, 147). Deficiency of calcium resembles that of boron in that both produce death of the terminal bud, but calcium shortage shows first at the tips of the young leaves, whereas boron deficiency is first seen as a light green color at the base of the young leaves, followed by a general breakdown (91, 93). Although the evidence is not conclusive, some indications of an association between the absorption of boron and that of calcium have been obtained in *Vicia faba* (162).

Mes (103) states that the symptoms of deficiency are most marked when vegetative growth is strongest, as has repeatedly been found with *Vicia faba* at Rothamsted. Attempts to replace boron by manganese were ineffective, though the manganese improved the vegetative growth and the green color. Boric acid or borax in small quantities has proved effective in ameliorating this deficiency, 5 pounds per acre being adequate in some cases (94). In Sumatra for years past boron compounds have been used as fertilizers in the ordinary commercial routine, their value as a preventive of disease being fully accepted (79, 104).

The tomato, another member of the Solanaceae, also needs boron for normal growth and for the setting and development of fruit (71, 151). The boron is apparently fixed in the tissues and cannot be used repeatedly by the plant, so a constant, though minute, supply is essential throughout the period of growth. When the main growing points are killed by deficiency, the buds are stimulated to develop till the boron supply is locally exhausted, when symptoms of degeneration again appear (146, 147).

Evidence for boron requirement of cereals is less definite, pos-

sibly because the minimum requisite amount is very low, enabling normal growth to be made under conditions which would induce disease in many other species which require larger amounts (138). The range of boron content in various species has been found to be lowest in barley, rye and wheat, from .1 to .3 mg. per kgm. dry weight, and highest in tomato, tobacco, potato, beans and peas, rising to a maximum of 18 mg. per kgm. dry weight (157).

Boron deficiency is accompanied by abnormal tillering in wheat, which can develop right up to the flowering stage in solutions containing no boron (111). The ears, however, either do not emerge or are badly developed and sterile, a condition which also appears in maize under similar circumstances (122). Oats may possibly need boron, though this is not fully established, as the yield of straw has been increased by boron manuring, but the yield of grain reduced (88). The incidence of fungus disease appears to be influenced by boron supply, and Eaton (38) found that *Erysiphe graminis* was abundant on boron deficient barley plants when it was absent from those receiving boron, whereas *Helminthosporium sativum* behaved inversely, the attack increasing in severity with increasing amounts of boron in the nutrient solution.

The injurious action of boron for citrus trees is widely recognized, and sufficient is contained in some irrigation waters to damage both citrus and walnut (143). Minute quantities are, however, essential and the anatomical and physiological changes induced by boron deficiency have been reproduced in controlled experiments (50, 53, 54). Here again the meristematic tissues are primarily affected, gum formation following. Abnormal carbohydrate accumulation occurs in the leaves, but as this excess is rapidly reduced if boron is supplied, it seems probable that an improvement in the conducting tissues plays an important part in the recovery of the plant.

Symptoms of boron deficiency manifest themselves later and develop more slowly during spring and autumn than in summer. This appears to be due to the reduced length of day (161), rather than to lower temperature. A certain correlation exists between the factors of boron and length of day as, with a variety of species, in the absence of boron the influence of length of day was found to be less striking than when boron was present, whereas

the boron deficiency symptoms were less pronounced under short day than under full day conditions.

Now that the signs of boron deficiency are becoming known, growers are beginning to attribute various cases of unhealthy or failing crops to this cause, and in many cases the trouble can be overcome by the use of small dressings of boron compounds. Sugar cane in water culture experiments exhibited depressed growth, distorted and chlorotic young leaves, and definite stem and leaf lesions in the absence of boron, normal growth being resumed if as little as .22 p. p. m.<sup>1</sup> of boron was added to the nutrient solution (99). The demands of strawberry are somewhat greater, varying according to the season of the year, 1 p. p. m. of boron preventing deficiency symptoms in spring, but not in summer. Cases have been observed in field conditions where deficiency of boron has definitely limited growth (57). Flax soon perished without boron, showing decay of the growing points of the shoots, and bad development of lateral rootlets (148). In water cultures the dry weights obtained were

|                       |          |      |          |
|-----------------------|----------|------|----------|
| With boron . . . . .  | 8.07 gm. | 100  | per cent |
| Without boron . . . . | .14 gm.  | 1.73 | per cent |

Under soil conditions, better growth occurred if boron was added, and the flower buds appeared earlier. Overtreatment with calcium- or manganese-carbonate on certain soils causes boron deficiency in flax, as it does with sugar beet, a condition that can be remedied by fertilizing with boron compounds (156).

Claims have also been made for the need of cotton (39), red clover (47), soybean (114), lettuce (86, 87), buckwheat (105) and blueberry (145) for boron, and doubtless other species will be added to the list as time goes on. Germination of maize and the early stages of growth in potatoes have also shown benefit from traces of boron (134). Care will be needed, though, not to attribute all obscure plant diseases to boron deficiency without adequate proof. Although it is perfectly clear that in many cases it is genuine damage due to lack of boron which is cured by the application of boron, we do not yet know whether in the soil there are other conditions causing unhealthiness in plants which are remedied

<sup>1</sup> Parts per million.

by treatment with boron. If this should be the case, the benefit of boron would be indirect, and not direct, but so far no evidence of this type of action has come to light, except in the possible case of rubidium injury in potatoes, which has been mitigated by the supply of available manganese and boron (56).

#### COPPER

For many years past the importance of copper has been recognized in connection with its function as the active principle of Bordeaux mixture, used for controlling fungal disease on important cultivated crops, as *Phytophthora infestans* on potato and *Peronospora viticola* on vines. This naturally led to enquiries as to the possible harmful action of the copper which falls to the ground during spraying, but all evidence in this respect has been negative, as the copper forms insoluble compounds in the soil with chalk, oxide of iron and alumina, and is, therefore, removed from the sphere of action (128).

More recently, the importance of copper in certain aspects of animal physiology has attracted much attention, and suggestions have been made that this element, in very minute quantities, may also be essential to plant life. This claim is by no means proved, but there are definite instances where copper has certainly improved growth in one way or another, though it cannot yet be regarded as "essential" in the true sense of the word. Far more critical work is necessary before copper can be considered on the same footing as boron and manganese.

The most spectacular work with copper was that of Allison, Bryan and Hunter (3) in 1927 on the sawgrass peat in Florida, when they found that the wholly unproductive soil could be made to produce excellent crops of lettuce, radish, turnips, rape, tomatoes, etc., by the addition of 30 pounds of copper sulphate per acre without any further manuring. Since then, various workers have found that copper is beneficial to crops grown on peat and muck soils (164), but, as in some cases similar effects can be obtained by the use of certain manures, as potash (167, 173), it is still a moot question as to whether the action of the copper is directly on the plant, or whether it acts indirectly by ameliorating some adverse condition in the soil. Allison (2) has attacked this problem and claims that in sugar cane normal development follows if copper is

introduced into the plant in other ways than by the roots, though this still does not eliminate the possibility of some action of copper on toxins absorbed from the soil. Whatever the explanation, the benefit itself cannot be questioned, and in Holland it is the recognized practice in reclamation of peat land to add 50 kg. per hectare of copper sulphate during the first year, as a preventive of what is known as "Urbarmachungskrankheit" or reclamation disease (cf. 20, 149, 171).

Certain types of chlorosis can be remedied by application of copper salts, either with the fertilizer at the roots or by spraying the leaves. The "frenching" or spotting of citrus leaves has been cured by spraying with Bordeaux mixture or by applying copper sulphate to the soil (117). Despite the increase in chlorophyll production, however, no copper was detected in the chlorophyll itself. Exanthema in pear trees is also attributed to copper deficiency, but there is no evidence as to whether the disease is due to the absence of copper *per se*, or to the presence of soil toxins, the effect of which is neutralized by the action of copper (118). Chlorosis of other deciduous fruit trees has also been cured by the use of  $\frac{1}{2}$ -2 pounds copper sulphate per tree, applied to the soil (5, 67). From another point of view the addition of copper sulphate to the usual fertilizers is said to improve the thickness and color of onion scales, though the results are not always consistent (77, 78).

Increased yields due to treatment with copper have been claimed in various quarters (135). Oats (19, 88), tomatoes (151), maize, sweet potatoes and beans (130) are among those mentioned and indicate the wide range of plants that apparently respond to copper, though in other cases no benefit was found with tomatoes (119) or buckwheat (105). It must, however, be remembered that an element may stimulate growth without being essential in the sense that in its absence vital aspects of growth are inhibited or seriously checked. More proof than this is needed, and certain investigators have applied more critical methods. Using water culture methods, Lipmann and McKinney (81) found that flax grew satisfactorily without copper till blossoming, but the amount of bloom was reduced and no capsules or seeds were produced. Barley also needed copper for seed formation, 1/16-1/18 p. m. of Cu in the nutrient solution being sufficient. Sommer (154), on the other hand, states that flax, tomatoes and sunflowers made little growth without cop-

per after the first week in nutrient solution. None of these workers has any clue to the rôle of copper, though it has been suggested that it may be auto-oxidant or catalytic in action. Among lower plants copper, as well as manganese and zinc, has been found to increase the growth of *Aspergillus flavus* and *Rhizopus nigricans*, better results being obtained from a combination of these elements than from each separately (84). The fat content was also increased but the proportion of nitrogen decreased by a very low optimum amount, the toxic limit soon being reached.

Although the claim for the essential nature of copper in the economy of plants cannot yet be substantiated, the evidence in hand is sufficiently encouraging to justify more extended investigations under strictly controlled conditions, for it is quite possible that copper may be essential for some plants or under certain conditions, and be unnecessary in other cases and for other species.

#### MANGANESE

The recognized importance of manganese for growth has encouraged work on the manganese content of plants, which varies considerably between species and also in a single species grown under different conditions. Lundegårdh (82) states that manganese is very slowly absorbed and that the total soil manganese has no great influence on the uptake. The addition of manganese sulphate to the soil may increase the manganese content of crops grown thereon (28), and with spinach a larger amount was found to be absorbed when the fertilizer was applied in several small dressings instead of all at once in a single treatment (108). This may imply that the sulphate remains available only for a short time and that repeated small applications enable the plants to utilize a larger proportion before the remainder becomes unavailable. The manganese content of plants grown in soils of varying acidity has been found to increase with the hydrogen ion concentration of the soil, due to a higher proportion of manganese in the soil water of acid soils (116).

Grasses vary considerably in their ability to take up manganese from the soil, the average content in one test varying from 207.5 mg. per kilogram for *Dactylis glomerata* to 78.1 mg. per kg. dry matter for *Poa pratensis*, while lucerne was lower than any grass, with only 46.6 mg. of manganese per kilogram (14). It is gen-

erally found that the proportion of manganese is higher in the leaves than in other parts of vegetables and fruits (126). Also, for any one species at any time the green leaves are always richer in manganese than etiolated, chlorotic leaves (11). The demands of species vary considerably, as is indicated by variation in the response of different crops to manganese fertilizers.

From the practical point of view the importance of manganese lies in its ability to prevent chlorosis and to increase the yield of crops. Manganese deficiency disease is usually manifested by a loss of green color, and is apt to be most marked on soils of high pH (30), rarely showing itself on acid soils. Heavy liming on some soils is, therefore, often followed by the appearance of trouble (80) due to the manganese in the soil being rendered unavailable for the plant, and where such liming is necessary for the production of certain crops the use of manganese fertilizers is essential (166).

Chlorosis due to manganese deficiency shows itself in characteristic ways. In tomato and cucumber plants the tops are first affected, the intravascular tissue of the leaves gradually changing from green to yellow while the veins and midribs remain green, producing a definitely mottled appearance. The general growth is weakened and the flower buds usually turn yellow and fall before opening (59). It appears that certain factors, as low temperature and slow growth, enable plants to withstand manganese chlorosis and that the trouble can also be overcome either by the addition of manganese compounds to the soil or by the correction of the soil reaction so as to make the manganese which is present available to plants (46). This correction can be made by increasing the acidity by the application of sulphur or ammonium sulphate, or by causing temporary water logging, in which the high degree of water saturation acts as a reducing agent (123). Many crops are improved either in health or yield by the application of manganese fertilizers, including blue lupins, soybeans (97), oats (29, 88), spinach, beets, blueberry (145), buckwheat (105), tomatoes (68, 129) and cucumbers, 100-150 lbs. per acre of manganese sulphate being effective with the latter (58). When all the tests are reviewed, however, it is evident that crops differ considerably in respect of their manganese requirements (115). In some cases, the reports



indicate that no benefit has been derived from manuring with manganese (64).

On the other hand, a type of chlorosis is induced also by excess manganese, as may occur with blue lupins on limed soil if there is a deficiency of iron (142), while poor growth of rice has been observed in the presence of an excess of soluble manganese in the soil (72). Chapman (25) has put forward the hypothesis that excess of manganese hinders the transport of iron to and from the leaf by converting it into an insoluble ferric form, thus causing chlorosis.

Citrus, sugar cane and tobacco are three other crops which react to deficiency of manganese. In Florida and California the growth of citrus and the quality of the fruit have been much improved and any tendency to chlorosis overcome on alkaline soils by applications of manganese salts (150). Haas (51) found that in acute cases of deficiency, citrus leaves absciss prematurely and the shoots die back. The roots remain healthy after deficiency symptoms are manifest in the shoots, suggesting that the roots absorb what manganese is available and retain it, not surrendering it to the stem and leaves unless more manganese is supplied.

Sugar cane exhibits the usual type of chlorosis due to manganese deficiency, and the yield and quality of the sugar is also affected (34, 98). The purity of the cane sugar is usually higher on soils with high manganese content (158), and there is also less brown-stripe disease on such soils (55). Sugar beet also shows increase in yield and sugar content if manganese deficiency is eliminated (48). The relation of sugar to manganese has been worked out in other species. Wheat, maize, lettuce and tomatoes grown without manganese have been found to be lower in reducing sugar and sucrose than those receiving manganese, indicating that the element plays some important part in sugar formation and sugar metabolism. In these experiments the manganese was injected straight into the stems, thus enabling both control and manganese plants to be grown in the same pots under identical conditions of nutrition and environment (107).

Apart from chlorosis, various other observations with relation to manganese have recently been made. Tobacco, while showing the usual deficiency chlorosis (93), is also very sensitive to excess, as plants growing on acid soils may be injured by toxic quantities



of soluble manganese (15). In such cases, phosphate fertilizers reduce the injury, probably by rendering the excess manganese inactive in the plant. Walnut yellows is a disease that is still little understood and for which manganese deficiency has been suggested as the cause. As, however, affected walnut leaves and bark contain a higher proportion of manganese than the healthy tissues, the cause cannot be attributed to manganese deficiency unless a considerable amount of the element that is present is for some reason or other unavailable for use in plant metabolism (52).

A most important aspect of manganese deficiency is its relation to grey-speck disease of oats and wheat. Inspired by Samuel and Piper (132), various investigators have obtained control of the disease by judicious use of manganese (33, 101, 163). Gerretsen, however, is now claiming that this does not represent the whole of the story, but that other factors of a bacterial nature combine with the manganese deficiency to cause the disease (45). Manganese deficiency in barley has been dealt with effectively by drilling  $\frac{1}{2}$  cwt. of manganese sulphate with the seed (144).

Claims have been made that small quantities of manganese stimulate the growth of various plants (136). As, however, this has been specifically claimed for plants grown under alkaline conditions it remains an open question as to whether a genuine stimulation occurs, or whether it is merely that an incipient manganese deficiency, causing reduction of yield without external symptoms of damage, is overcome by the application of manganese salts, resulting in an improvement in growth which suggests stimulation. Seeds of chickpea and peanut treated with .5 per cent solutions of manganese sulphate before sowing have been found to grow faster than control seeds for the first 10–20 days, this being attributed to the effect of the salt in accelerating enzyme activity during mobilization of food reserves and early stages of plant growth (172).

A certain amount of work has been continued with cryptogams and simpler phanerogams. Traces of manganese are beneficial to yeast, increasing the dry weight (85), though the toxic limit is soon reached, resulting in decreased growth or death of the cells. Further claims are made for the importance of manganese for *Aspergillus niger*, for which it appears to be essential for normal growth and sporulation (155). Hopkins (60, 61) found that the green alga *Chlorella* made no growth without manganese and sug-

gests that the element functions physiologically in an indirect manner by its action on the state of oxidation of iron, so that sufficient manganese must be present to ensure the oxidation. The evidence in regard to *Lemna* is conflicting, as workers who originally stated that manganese had no beneficial effect (27), have repeated their work and now state that minute traces in the nutrient salts must have been overlooked, and that it appears that *Lemna major* does need a trace, though 1 : 300,000,000 is sufficient to give good growth (26). Here again the toxic limit is very soon reached, 1 mg. Mn per litre being too high a concentration for optimum growth (131).

#### ZINC

The essential nature of zinc in fungus nutrition has been claimed for many years, since Raulin (125) worked in 1869 with *Aspergillus niger*. After much controversy, the general opinion is that this view is correct, and various workers have put forward further proof in the last five years. Steinberg (155) states that normal growth and sporulation of *Aspergillus niger* can occur only in the presence of several minor elements, including iron, copper, manganese and zinc. The dry weight of yeast (85) is also increased by zinc, though, as usual, too heavy doses are toxic.

Among the higher plants claims are made for the value of zinc from two aspects—as a stimulant to the growth of certain crops, and as a specific against certain diseases. McHargue and Shedd (88) obtained increases in straw and grain of oats by the addition of traces of zinc to sand cultures, whereas Scharrer and Schropp (140) found greater stimulation with other cereals and peas than with oats. Buckwheat and flax have also been improved by zinc in some circumstances as, for instance, where flax was grown on heavily limed acid soil (73).

The more important aspect is in relation to plant diseases which are attributed to a deficient supply of zinc. Frenching or mottle-leaf of citrus, little-leaf of fruit trees (96), court-noué of vines (36), pecan rosette (42) and bronzing of tung trees (113) have all been successfully treated by zinc sulphate, leading to the assumption that zinc is essential for certain metabolic functions and that a deficiency of the element hinders normal development. Applications of zinc sulphate to the soil, varying from .25 to 15 lbs. per tree, according to the type of citrus, have caused marked im-

provement in badly frenched trees (23), but this method sometimes fails. Spraying with solutions of zinc sulphate is more generally successful (159) and sometimes direct injection into the tissues is satisfactory (95). According to Dufrenoy and Reed (37) zinc, as well as iron, has a specific effect on leaf assimilation, mottle-leaf being a pathological symptom indicating an interruption of equilibrium between the cytoplasm and its inclusions. The provision of zinc to affected plants increases chlorophyll production and photosynthetic activity. A significant point is that zinc is present in the cells of treated, but not untreated, orange trees, leading to the direct association of zinc with recovery of the plant.

Little-leaf or rosette of fruit trees is characterized by the production of numbers of abnormally small leaves, and the value of zinc as a corrective is acknowledged from many quarters. Opinions differ as to the best method of application, and soil applications, injection into the tissues (35) and spraying (121) or dipping all have their advocates. While it is probable that little-leaf of fruit trees is a symptom of an inadequate supply of zinc for normal metabolism, Chandler, Hoagland and Hibberd (24) point out that the trouble may not be due to zinc deficiency only, as large woody perennials grown on the same soil as the fruit trees are also susceptible, while annual plants are generally free from attack. They suggest the possibility that zinc may aid in the precipitation of toxic substances formed by certain soil bacteria, and that the beneficial action of zinc may thus be indirect rather than direct.

Though the beneficial effect of zinc in these various types of abnormal development cannot be denied, no definite proof yet exists that zinc is essential for normal development of higher plants. Such proof can be given only by experiments in which plants are grown from seed in the entire absence of zinc, as has been done with boron, copper and manganese. If little-leaf, frenching, etc., could then be produced artificially, the practical results already available would provide a most valuable weight of evidence in support of the hypothesis.

#### OTHER ELEMENTS

Up to the present, definite evidence of the essential nature of "minor" elements has been established only for the four elements already examined. A considerable amount of work has been done

with others with the same objective, but so far with very little success. The much-tested fungus, *Aspergillus niger*, has shown some stimulation with very low strengths of various elements (124), higher concentration being very toxic. Other elements exhibit toxicity and among the halogens fluorine is the most poisonous. With higher plants only indifferent or toxic action has so far been proved with such elements as bromine (168), fluorine (105, 112, 133), molybdenum (139), selenium (65, 66, 76), uranium (7), thallium (62, 83, 92), caesium (4), palladium, beryllium and zirconium (6, 22). Rubidium is usually found to be toxic or indifferent (4, 22) though Loew had earlier claimed that in small amounts it benefited Chinese cabbage, barley and spinach. Rather more evidence of occasional stimulation occurs with a few other elements, described below in more detail.

#### ALUMINUM

Toxicity of aluminum is repeatedly being shown (41) but there is no evidence that small quantities of the element are essential for growth. The beneficial effects occasionally recorded are generally due to soil reaction caused by the aluminum liberating supplies of definitely necessary elements, such as iron (127), of which scarcity causes chlorosis.

#### ARSENIC

Arsenic is also noted for its toxicity (31, 120), but in conjunction with other minor elements it has been found to increase the frost resistance of young maize plants under certain cultural conditions (122).

#### BARIUM, LITHIUM, STRONTIUM AND CHROMIUM

The first three may possibly play some part in the metabolism of sugar cane, as they have all been found in very productive soils, while they were absent in poor soils. Strontium, with chromium and zinc, may also exercise some inhibitory action on diseases of sugar cane, which is interesting in view of the stimulation exercised by strontium on *Aspergillus niger* in high concentrations (124). In some areas, the proportion of brown-stripe disease of sugar cane varies inversely with the amount of chromium present in the soil (55).

## CADMIUM

It has been claimed that cadmium stimulates oats, rye, wheat and barley grown in sand or water cultures, the concentration varying with the species, but maize was not found to respond. The degree of stimulation was less than that obtained with equivalent amounts of zinc (140). Low concentrations of cadmium have also been found to stimulate growth of *Aspergillus niger*, high strengths being toxic (124).

## COBALT

Cobalt is a widely distributed constituent of plants, occurring in small quantities in many species. No relation has yet been proved (12) between plant growth and the presence of cobalt, though some workers have suggested that both cobalt and nickel may act as catalyzers in the living plant (10). Cobalt in great dilution has been found to act favorably on *Aspergillus*, though the effect is not so clear as with nickel (110).

## IODINE

While it is generally recognized that iodine is toxic in stronger concentrations (109, 170), opinions differ as to its action in great dilution. Whereas Meyer claimed that iodine is essential for the best growth of buckwheat (105), Cotton states that it exerts no beneficial action on that species even in great dilution (32). Potassium iodide has been found to improve lettuce, cucumber and tomatoes, causing quicker growth and preventing crimping in lettuces and stem-rotting in tomatoes (75).

## MERCURY

Advantage is taken of the toxicity of mercury to control such diseases as potato-scab, at the risk of reduction of crop (40, 90). The possibility also exists that mercury may precipitate the toxins causing little-leaf of fruit trees (24), but no directly beneficial effect upon growth has been shown.

## NICKEL

Nickel is so widely distributed that it may almost be regarded as a normal constituent of plant tissues (100), but it has not yet been proved that it plays any essential part.

## TUNGSTEN

Stimulation of the early growth of seedlings of various cereals and peas has been observed with low concentrations of tungsten in the form of sodium tungstate, the beneficial strength varying considerably with the species (139).

## SUMMARY

The available evidence makes it quite clear that small amounts of boron and manganese are essential to the growth and health of many, if not all, species of plants. Copper and zinc have also been found to be necessary in many cases, though up to the present it is uncertain whether this need is universal. Apart from these four elements, isolated cases only of improvement due to traces of other minor elements have as yet been established. It may be, however, that specific elements are necessary for specific plants, and it is possible that the conclusive evidence already obtained with boron and manganese may further the opening of a wide field of investigation which may lead to results of far-reaching importance from scientific and economic standpoints.

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## GENETICS OF POLYPLOIDY\*

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Changes in chromosome number directly affect gene inheritance and, in turn, the morphology and physiology of cells and of plants. Were additional proof of the gene-chromosome law of heredity necessary, the genetical behavior of polyploids affords a most potent experimental verification, for with a change only in chromosome number, a direct, corresponding change in inheritance is made manifest.

For the general botanist, changes in chromosome number may conveniently be reviewed in their results on (1) gene and character inheritance and (2) general effects on cell and plant morphology, physiology, ecology and evolution. Since a detailed account of the cytogenetical aspects of polyploidy may be found in Sansome and Philp's book (45) on "Recent Advances in Genetics," in Darlington's "Recent Advances in Cytology" (15) and in Sharp's last edition of "Introduction to Cytology" (46), only the broader and newer points will be developed in this review.

The following brief glossary of terms will probably be of assistance to the lay reader.

1. Change in chromosome number in only one pair of the diploid.  
Monosomic =  $2n - 1$  (also called haplo-forms).  
Trisomic =  $2n + 1$  extra chromosome.  
Primary =  $2n + 1$  (extra is complete homologue of one pair).  
Secondary =  $2n + 1$  (extra has two similar arms).  
Tetrasomic =  $2n + 2$  (both extras homologous with one original pair).  
Gene conditions—Quadruplex *AAAA*, triplex *AAAa*, duplex *AAaa*, simplex *Aaaa*, nulliplex *aaaa*.
2. Change in chromosome number of all sets or pairs.  
Heteroploid (polyploid)—a form with chromosome number other than the true haploid (monoploid) or diploid number.  
Euploid—an exact multiple of the haploid (triploid, tetraploid, penta-, hexa-, hepta-, octoploid, etc.).  
Autoheteroploid (autopolyploid)—a multiple chromosome complement of a single kind of the haploid set. An autotetraploid, for example, has four similar chromosomes in each set.  
Alloheteroploid (allopolyploid)—a multiple chromosome complement of dissimilar sets of chromosomes.  
Aneuploid—a chromosome number other than an exact multiple of the haploid.  
Hypoploid—a little lower than some multiple.  
Hyperploid—a little higher than some multiple.

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Strictly speaking, monosomics and even trisomics should not, perhaps, be included under the term polyploidy but some of their cytogenetical reactions, especially of the trisomics, often prove useful in understanding the more complex behavior of the true polyploids.

#### *Gene Segregation in Polyploids*

Gene distribution is directly affected by the chromosome behavior in triple (trisomics or triploids), quadruple (tetrasomics or tetraploids) or in the higher multiple sets, so that different genetic ratios emerge from hybridization experiments. Chromosome behavior in triploids is reasonably similar in most species, but in tetraploids there are differences in disjunction among the various species that influence fertility (4, 5, 16, 22, 24, 32, 37, 40, 49).

#### *Gene Ratios in the Triple Condition*

Triploids ordinarily arise from the union of gametes containing  $2n$  and  $n$  chromosomes. They have been produced experimentally by crossing tetraploids and diploids, better success usually following use of the  $4n$  as the maternal parent. In the reciprocal cross,  $2n$  pollen tubes of the tetraploid are not particularly adapted to germination or growth in the  $2n$  stylar tissue.

Meiotic divisions in triploids are very irregular. Sometimes trivalents are formed but very frequently a bivalent and a univalent are observed. In either case, unbalance of chromosomes occurs and the percentage of abortive gametes is high. The functional gametes of a triploid are usually those containing the  $n$ ,  $2n$  or the  $n+1$  and  $n+2$  number of chromosomes. The progeny, likewise, show a high frequency of  $2n+1$  forms plus a small percentage of tetraploids. The characteristic sterility of triploids seems largely to be due to the unbalance of genes in the gametes.

Because of the great amount of sterility in triploids it is not profitable to discuss genic inheritance. Better results come from a study of trisomics where only one chromosome at a time is added.

The classical research with *Datura* trisomics (5, 6, 8) is so well described in text books that details will be omitted in this review. The same is true for the well known *Drosophila* investigations of trisomics, as well as those in maize (34, 41, 42), tomato (28, 29, 30) and *Nicotiana* (12).



Trisomics ordinarily arise from triploids or from diploids through non-disjunction of one bivalent. With three homologous chromosomes,  $X$ ,  $X'$  and  $x$ , random pairing should give the gametes  $XX'$ ,  $Xx$ ,  $X'x$ ,  $X$ ,  $X'$  and  $x$ . In a duplex trisomic ( $AAa$ ), the gametic ratio of genes should be  $1AA:2Aa:2A:1a$ , whereas in a simplex form ( $Aaa$ ) the ratio becomes  $2Aa:1aa:1A:2a$ . But these ratios are never realized in breeding experiments because the male gametes with the extra chromosome are rarely functional in competition with normal microspores. Even the female gametes with the extra chromosome are usually represented much less frequently than 50 per cent.

Cytologically, chromosome disjunction of a trisomic is typically a random  $2 \times 1$  affair, and only two of the chromosomes synapse or crossover at any one region. Hence, some of the progeny of a self-fertilized plant are disomic and some trisomic. For example, in primary *Datura* (6, 8) trisomics an average of only 24 per cent of the progeny is trisomic (range from 11 to 32 per cent). When once the percentage of trisomics in the progeny is determined, it is usually discovered that gene segregation follows random assortment of the three chromosomes, modified by any crossing-over in the six strand (chromatid) stage. Accordingly, the gene ratio is affected by the distance of the genes from the spindle-fiber-attachment. The recent work with maize trisomics is particularly in accord with this concept (34, 42).

#### *Gene Ratios in the Quadruple Condition*

More regular genetic data are often obtained from tetraploids than triploids due to a better balance of the chromosomes and, hence, a higher degree of fertility. In many tetraploids there is a high frequency of bivalent chromosome association at diakinesis, although some sterility enters whenever a  $3 \times 1$  disjunction occurs. It is for this reason that the fertile progeny of self-fertilized tetraploids consists largely of  $4n$  plants and, hence, genetic data from  $4n$  hybrids can be utilized to trace chromosome and gene distribution. This is particularly true of some tomato tetraploids (32), but with maize tetraploids there are greater cytological irregularities (40).

Tetraploids arise either naturally or artificially. They may come from the occasional  $2n$  gametes of triploids. Doubling may also occur in somatic tissue of the diploids. Artificially, they are easily



made in certain species of the *Solanaceae*, like the tomato, by the decapitation technique (24, 31, 32, 53) where somatic doubling of nuclei takes place in callus tissue. Such tetraploids are valuable for controlled experiments involving known genetic constitutions.

Whenever doubling occurs, two extreme situations may be encountered. In one, the chromosomes may be completely homologous or nearly so. This is the autotetraploid. An extreme case of this is the  $4n$  form of *Lycopersicum esculentum* derived from the haploid *via* the diploid (31, 32). This is an absolutely homozygous tetraploid, the four chromosomes in each of the 12 sets being identical since each single chromosome came from the haploid originally. This tetraploid is the most sterile of all tomato tetraploids. Autotetraploid *Datura* plants, on the other hand, are reasonably fertile, despite the fact that their chromosomes associate as quadrivalents, allowing irregular disjunction (4, 5, 9).

The other extreme is that of the allotetraploid where two of the chromosomes in any set are very different from the other two. As a result, there is a minimum of quadrivalent prophase association and, subsequently, a high degree of bivalent chromosome pairing at diakinesis, giving fairly regular disjunction which results in a large percentage of functional diploid gametes. In most allotetraploids, however, there is some degree of quadrivalent association of the chromosomes.

Allotetraploids are more fertile than autotetraploids. In the latter, only autosyndetic pairing of similar chromosomes is to be expected whereas in allotetraploids both autosyndesis and allosyndesis (pairing of dissimilar chromosomes) may occur, depending on the phylogenetic relations of the plants involved in the formation of the tetraploid. In an allotetraploid derived from two species, such chromosome similarity, as evidenced by pairing, may serve as a basic factor in determining taxonomic relationships. In extreme cases of allotetraploidy, where no allosyndesis occurs, the conclusion that the two forms are true, legitimate, genetic species, seems to be justified.

Genetically, tetraploids (or tetrasomics) may consist of the following genotypes:  $AAAA$ ,  $AAAa$ ,  $AAaa$ ,  $Aaaa$ , or  $aaaa$ , the letters representing genes in any one set of chromosomes. In many respects the duplex condition,  $AAaa$ , serves well to test the gene-chromosome relations.

Given four homologous chromosomes in any one set such as may be found in an autotetraploid or an allotetraploid where the genetic differences are not too great, there are four possible fundamental methods of synapsis and subsequent disjunction. When measured by genetic tests of  $4n$  hybrids, such as  $AAaa$ , certain  $2n$  gametes and  $4n$  progeny result which may be determined by  $F_2$  or back-cross (to recessive  $4n$  types, since a  $4n \times 2n$  cross is ordinarily sterile) experiments. The four methods are illustrated in Table 1:

TABLE 1  
Summary of four possible methods of chromosome behavior in tetraploids

| Method of pairing                          | Bivalent association      | 2n Gametes |      |      | $F_2$ ratio<br>$A : a$ | Back-cross ratio<br>$A : a$ |
|--|---------------------------|------------|------|------|------------------------|-----------------------------|
|  |                           | $AA$       | $Aa$ | $aa$ |                        |                             |
| 1. Preferential (autosynopsis) .....       | $\frac{A}{A} \frac{a}{a}$ | 0          | 1    | 0    | 1:0                    | 1:0                         |
| 2. Preferential (allosynopsis) .....       | $\frac{A}{a} \frac{A}{a}$ | 1          | 2    | 1    | 15:1                   | 3:1                         |
|  | $\frac{A}{a} \frac{a}{A}$ |            |      |      |                        |                             |
|  | $\frac{a}{a} \frac{A}{A}$ |            |      |      |                        |                             |
| 3. Random assortment of 4 chromosomes ...  | $\frac{A}{A} \frac{a}{a}$ | 1          | 4    | 1    | 35:1                   | 5:1                         |
|  | $\frac{A}{a} \frac{A}{a}$ |            |      |      |                        |                             |
|  | $\frac{a}{a} \frac{A}{A}$ |            |      |      |                        |                             |
| 4. Random assortment of 8 chromatids ..... | See Table 3               | 3          | 8    | 3    | 20.8:1                 | 3.7:1                       |

Method 1. Preferential pairing of similar chromosomes (autosynopsis). When two chromosomes of a tetrasome are very different genetically from the other two, or when a true allotetraploid is involved, and pairing is conditioned by gene-by-gene attraction, it is apparent that the two similar chromosomes should synapse. If, then, disjunction is from a bivalent condition, as is often the case, all the diploid gametes should be alike ( $Aa$ ) and the hybrid should breed true. Such is rarely the case, but it has been reported in tetraploids from very wide species or genus crosses, although the evidence rests on genetic, rather than cytological, grounds. For example, in the allotetraploid *Raphanus-Brassica* hybrids (25, 26), in *Spartina Townsendii* (23) and in *Primula kewensis* (37, 39),

chromosome pairing is largely in the bivalent condition and these hybrids are very fertile and breed approximately true. In the latter hybrid, the parental *P. verticillata* chromosomes generally pair among themselves as do the *P. floribunda* chromosomes. However, one quadrivalent set is often present, indicating a certain relationship between the two species. A similar situation is found in the species tetraploid *Nicotiana digluta* (11) which arose from 12 *N. glutinosa* and 24 *N. tabacum* chromosomes that were doubled, giving 36 pairs and making a true breeding form with regular meiotic behavior.

The above method is not found in true autotetraploids, even when the two chromosomes are markedly different genetically from the other two. Here there is quadrivalent prophase association with crossing-over between the four chromosomes (or eight chromatids) allowing for chromosome or chromatid segregation.

Method 2. Preferential pairing of dissimilar chromosomes (allosyndesis). There seems to be no apparent reason for such a condition if pairing is instituted by a gene-by-gene attraction. However, some earlier data on *Primula* (20) tetraploids were fitted to such an hypothesis, resulting in 15:1  $F_2$  or 3:1 back-cross ratios of dominant to recessive types. Later these meager data were better explained on another basis (method 3 below) by Muller (35).

Method 3. Random assortment of four chromosomes. If, in a  $4n$  *AAaa* hybrid, the four chromosomes of any one set synapse at prophase in a tetravalent condition and later emerge as bivalents, a  $1AA + 4Aa + 1aa$  assortment of diploid gametes results. This gives a 35:1  $F_2$  phenotypic ratio or a 5:1 back-cross ratio of dominants to recessives (Table 2). Practically all tetraploid data thus far reported have been fitted to such an hypothesis. The early *Datura* experiments (9) seem to afford a close fit to expectation for the *Pp* (stem color) genes and to a lesser extent for the *Ss* (spiny capsule) genes. In the *Primula sinensis* tetraploid the data fit closely to this method (49, 54).

Method 4. Random assortment of eight chromatids. Modern cytological and genetical research has demonstrated beyond reasonable doubt that in a diploid the two homologous chromosomes pair in early prophase and then undergo a longitudinal split, giving four chromatids. It is at this stage that crossing-over occurs.

TABLE 2  
*Tetrasomic inheritance in hybrid progenies, selfed and back-crossed to recessive*

*recessive*

| 4n hybrids                                 | Mated with | Progeny |      |      |      |      | Ratio<br>A:a |
|--|------------|---------|------|------|------|------|--------------|
|  |            | AAAA    | AAAa | AAaa | Aaaa | aaaa |              |
| I. With random assortment of 4 chromosomes |            |         |      |      |      |      |              |
| AAAa                                       | Self       | 1       | 2    | 1    |      |      | 1:0          |
|  | aaaa       |         |      | 1    | 1    |      | 1:0          |
| AAaa                                       | Self       | 1       | 8    | 18   | 8    | 1    | 35:1         |
|  | aaaa       |         |      | 1    | 4    | 1    | 5:1          |
| Aaaa                                       | Self       |         |      | 1    | 2    | 1    | 3:1          |
|  | aaaa       |         |      |      | 1    | 1    | 1:1          |
| II. With random assortment of 8 chromatids |            |         |      |      |      |      |              |
| AAAa                                       | Self       | 225     | 360  | 174  | 24   | 1    | 783:1        |
|  | aaaa       |         |      | 15   | 12   | 1    | 27:1         |
| AAaa                                       | Self       | 9       | 48   | 82   | 48   | 9    | 21:1         |
|  | aaaa       |         |      | 3    | 8    | 3    | 11:3         |
| Aaaa                                       | Self       | 1       | 24   | 174  | 360  | 225  | 559:225      |
|  | aaaa       |         |      | 1    | 12   | 15   | 13:15        |

Undoubtedly, the same thing prevails in tetraploids where the eight chromatids of the four chromosomes in any tetrasomic set could be associated at prophase. Crossing-over between chromatids occurs as in the diploid. If any one gene locus is far enough removed from the spindle-fiber-attachment, such crossing-over may affect every such gene locus among the eight chromatids. This would approach a random interchange among the eight chromatids, the maximum state being a wholly random assortment of the eight. If a gene is borne near the spindle-fiber-attachment there would be less chance of an interchange separating sister chromatids. In this case, the genes would be assorted as in Method 3.

Mathematically, a random assortment of eight chromatids in a 4n hybrid, AAaa, results in a 3AA+8Aa+3aa proportion of diploid gametes (Table 3). This gives a 20.8:1 F<sub>2</sub> or an 11:3 back-cross ratio of dominants to recessives as a maximum. With less crossing-over, such as would hold for genes nearer the spindle-fiber-attachment, an approach to the 35:1 F<sub>2</sub> ratio as a limit would result. In other words, the percentage of recessive gametes from an AAaa form varies between 16.7 and 21.4 per cent, depending upon the amount of crossing-over between the spindle-fiber-attachment and the gene in question.

TABLE 3  
Gene relations under complete random assortment of eight chromatids in a tetrasomic set

| Bivalents                            | Summary of daughter cell constitution | Gametes (2n) |    |    |
|--------------------------------------|---------------------------------------|--------------|----|----|
|                                      |                                       | AA           | Aa | aa |
| 1 .... $\frac{AA}{AA} \frac{aa}{aa}$ | 1 AA-AA                               | 1            |    |    |
| 1 .... $\frac{AA}{aa} \frac{aa}{AA}$ | 16 AA-Aa                              | 8            | 8  |    |
| 1 .... $\frac{AA}{aa} \frac{AA}{aa}$ | 12 AA-aa                              |              | 12 |    |
| 8 .... $\frac{AA}{Aa} \frac{Aa}{aa}$ | 24 Aa-Aa                              | 6            | 12 | 6  |
| 8 .... $\frac{AA}{Aa} \frac{aa}{Aa}$ | 16 Aa-aa                              |              | 8  | 8  |
| 8 .... $\frac{AA}{aa} \frac{Aa}{Aa}$ | 1 aa-aa                               |              |    | 1  |
| 8 .... $\frac{Aa}{Aa} \frac{Aa}{Aa}$ |                                       |              |    |    |
| Total .....                          |                                       | 15           | 40 | 15 |
| Ratio .....                          |                                       | 3            | 8  | 3  |

There are only a few recent experiments that have been fitted to such an hypothesis. These deal with tetraploids in *Lycopersicum* (32, 44) and *Rubus* (14). For illustration, a summary of the tomato data appears in Table 4.

Each of the gene ratios in Table 4, representing six of the twelve different chromosomes of the tomato, shows a distinct tendency to be less than 35:1 in  $F_2$  progenies. This is equally true of the autotetraploid, *L. esculentum*, and the allotetraploid represented by the species cross, *L. esculentum*  $\times$  *L. pimpinellifolium*. The consistency of the data affords strong evidence against a mere random assortment of the four chromosomes and points to a chromatid assortment. The latter is apparently made variable by the amount of crossing-over between the spindle-fiber-attachment and the gene in question. For example, the *Yy* genes approach most closely the 35:1 ratio of chromosome assortment, indicating that these genes are close to the fiber-attachment. The *Rr* genes approach very

TABLE 4  
Tetraploid  $F_2$  segregations of genes carried on six different chromosomes

| Tetraploid, duplex hybrids  | D    | d   | R    | r  | Y   | y  | C    | c  | A    | a  | B   | b |
|---|------|-----|------|----|-----|----|------|----|------|----|-----|---|
| <i>L. esculentum</i><br>(Sansome, 1933) .....   | 995  | 43  | 647  | 26 | 554 | 17 |      |    | 172  | 4  | 207 | 8 |
| <i>L. pimpinellifolium</i><br>$\times$ <i>L. esculentum</i><br>(Lindstrom and<br>Humphrey, 1933) .... | 883  | 28  | 376  | 19 | 382 | 13 | 545  | 33 |      |    |     |   |
| New data—1935 .....   | 772  | 37  | 165  | 10 |     |    | 769  | 59 | 992  | 37 |     |   |
| Total .....   | 6250 | 108 | 1188 | 55 | 936 | 30 | 1314 | 92 | 1164 | 41 | 207 | 8 |
| Ratio .....   | 25   | 1   | 22   | 1  | 31  | 1  | 14   | 1  | 28   | 1  | 26  | 1 |

closely the 21:1 ratio of a wholly random assortment of eight chromatids, indicating that they are farther removed from the attachment. The  $Cc$  genes in Table 4 appear to favor an allosyndetic (Method 2) pairing, but they should not be considered as critical evidence because of the uncertainty of classification of the characters involved (tomato *vs.* potato leaf) in the young plant stage when some of the counts were made. With this exception, the  $4n$  tomato data seem to follow a cellular mechanism of gene distribution that is based on chromatid segregation, modified by some degree of crossing-over.

It is worth pointing out that the autotetraploid tomato hybrids in Table 4 give approximately the same genetic results as the more allotetraploid forms produced by crossing the wild and the domesticated species. In this latter allotetraploid form with 48 chromosomes, two chromosomes in each set of four are identical *esculentum*, and two are identical *pimpinellifolium* chromosomes. This is true because the tetraploid arose from a  $2n F_1$  hybrid of the two species in which there were twelve pairing sets of chromosomes. The doubling of the  $2n F_1$  was done artificially under controlled conditions (32). Thus, in any of the 12 tetrasomes there are two wholly identical *esculentum* and two identical *pimpinellifolium* chromosomes with every gene alike among the two, and with hundreds of allelomorphic differences between the genes of the two species.

It should be noted, however, that there must be many more genic similarities than dissimilarities in the two species making up the

tomato allotetraploid because all the chromosomes of the diploid  $F_1$  species-cross synapse, albeit incompletely. The same homology is undoubtedly carried over into the tetraploid hybrid. Hence, this partially allotetraploid form undergoes a chromosome synapsis similar to the autotetraploid, resulting in a corresponding genetic behavior.

An interesting and critical point in differentiating between chromatid and chromosome segregation in tetraploids arises in observing the genetic behavior of a triplex condition of the genes, such as  $AAAa$ . If these genes followed chromosome segregation (Method 3), the bivalent gametes could only be  $AA$  or  $Aa$ , and no recessive character would emerge in the progeny. But with chromatid segregation, three kinds of gametes are possible,  $AA$ ,  $Aa$  and  $aa$  in the proportion of 15: 12: 1 (Table 2).

Special cases of polyploid genetics, due to peculiar cytological conditions, may arise to give ratios intermediate to those shown in Table 4. A recent example of this is the tetraploid hybrid of maize and perennial teosinte (13, 17, 18). In one such case (13) the hybrid had 20 maize and 20 teosinte chromosomes, giving regular meiosis and good pollen. A much smaller percentage of recessive (waxy) gametes was found than the 16.7 per cent expected with random assortment of four chromosomes or the 21.4 per cent due to random assortment of eight chromatids. This was accounted for by a greater autosyndetic than allosyndetic pairing. A formula was devised for measuring the degree of autosyndesis as follows:

$$t = \frac{1 - 6x}{1 - 2x}$$

where  $x$  = the ratio of recessive gametes and  $t$  = the 'coefficient of autosyndesis.' From this formula a  $t$  of  $-1$  means complete allosyndesis,  $t = 0$  means random pairing and  $t = +1$  means complete autosyndesis. Collins and Longley (13) found values of .90 in  $F_1$ , .77 in  $F_2$  and .74 in  $F_3$  material, all indicating a high degree of autosyndesis for the waxy gene-containing chromosomes. Evidently, the maize chromosomes pair preferentially in this genus cross. Genetically the same situation was reported by Emerson (17) who had strong evidence that the teosinte chromosomes of a maize-teosinte hybrid paired preferentially.

*Gene Ratios in Higher Polyploid Cases*

There is no critical genetic research with the higher polyploids. In the octoploid *Dahlia* case (27) Lawrence has reported on two sets of genes but both really show tetraploid inheritance.

*Linkage in Polyploids*

Several cases of linked inheritance have been reported in polyploids (44, 54), but the complexities of the situation render the subject a highly technical one which has no place in a general review. De Winton and Haldane (54) report that the linkage intensity in  $4n$  *Primula sinensis* is approximately the same as in the diploid form. Sansome found the same condition in the tomato (44). For reference, Haldane's (21) statistical treatment of theoretical linkage intensities will serve the specialist.

*Inbreeding and Random Mating in Polyploids*

The genetical behavior of polyploids in later generations, following various systems of mating, is reported in two papers, one by Haldane (21) and the other by Bartlett and Haldane (2). In the first paper, the gametic series to be expected from various types of heterozygous autopolyploids are listed, together with the effects of self-fertilization and random mating on populations. In the second paper, formulae and rates of decrease of heterozygosis under brother and sister matings are given.

*General Effects of Polyploidy*

Increase in chromosome number affects the organism in several general respects. Cell size is directly modified as may be seen by a typical set of polyploid data of the tomato (Table 5). Cell volume is approximately doubled or slightly less than doubled, with chromosome doubling. In *Funaria* tetraploids, Wettstein (51, 52) reports that cell volume increased at least 1.7 times that of corresponding diploids.

It is well known that plant size is also affected. The effect of  $n$ ,  $2n$  and  $4n$  chromosomes on tomato size is well illustrated in Figure 1 which is a typical case and is particularly convincing because the doubling was done asexually under complete experimental control (decapitation technique).



TABLE 5

*Linear micromorphological measurements of tomato polyploids in microns*  
From Lindstrom and Humphrey (32)

|  | Pollen diameter | Cell size Diakinesis | Nuclear size Diakinesis |
|--|-----------------|----------------------|-------------------------|
| <i>L. esculentum</i> — <i>n</i> .....        | 25.7            | 13.4                 | 7.7                     |
| <i>L. esculentum</i> — <i>2n</i> .....       | 25.7            | 16.5                 | 9.0                     |
| <i>L. esculentum</i> — <i>4n</i> .....       | 30.0            | 21.8                 | 13.1                    |
| <i>L. pimpinellifolium</i> — <i>2n</i> ..... | 21.6            | 16.7                 | 8.9                     |
| <i>L. pimpinellifolium</i> — <i>4n</i> ..... | 27.1            | 21.9                 | 12.2                    |

*Cell volumes of tomato polyploids in cubic microns*  
From Humphrey (22)

|                 | Pollen | Pollen mother cell at Diakinesis | Nucleus at Diakinesis | Nucleolus at Diakinesis |
|-----------------|--------|----------------------------------|-----------------------|-------------------------|
| <i>n</i> .....  | 8693   | 1251                             | 228                   | 3.6                     |
| <i>2n</i> ..... | 8693   | 2356                             | 379                   | 8.1                     |
| <i>4n</i> ..... | 14040  | 5289                             | 899                   | 14.0                    |

In *Datura* and maize trisomics, experienced observers can detect the phenotypic variations caused by each extra chromosome. Evidently each chromosome carries genes that influence the gross morphology in plus and minus directions. Another influence of extra chromosomes may be seen in the histological study of *Datura* trisomics (7, 47). Here the addition of any one of the 12 chromosomes had a detectable effect on the anatomical pattern of petiole structure.

Sexual differentiation due to tetraploidy has been reported in *Sphaerocarpus* (1) where spore dyads (instead of the regular tetrads) give rise to diploid gametophytes. These are functionally females although containing both X and Y chromosomes.

Physiologically, there are striking effects of polyploidy on the plant as a whole. Growth rate is usually slowed down, the tetraploid being slower than the diploid and the diploid slower than the haploid. Regeneration (from cuttings or from callus) is, likewise, faster in the haploid than the diploid.

In this connection it is worth noting that in some species there seems to be a relationship between polyploidy and growth habit.

The diploid (20 chromosomes) *Euchlaena mexicana* is an annual, while the tetraploid *E. perennis* (40 chromosomes) is a perennial. Longley (33) has shown that seven annual species of *Sorghum* have 10 as the haploid number whereas the perennial species, *S. halepensis*, contains 20 chromosomes and a closely related genus, *Sorghastrum* with 20, is also perennial.

Differential ecological relations in polyploid series have recently been reported in *Dianthus* (43). In this genus Rohweder finds that diploid species possess a more limited longevity and poorer adaptability than tetraploid or hexaploid forms. Tetraploid carnations were found to surpass considerably the diploids in their adaptation to lime. The forms with larger chromosomes withstood poor soil conditions better than those with small chromosomes.

The real causes for these general increases in cell or plant size and in physiological response are not fully understood. Critical research on these points is highly desirable, particularly in differentiating between the action of the genes themselves and the gene chromatin. In a tetraploid, for example, not only are the genes doubled but also the chromatin. The latter substance is largely composed of chromomeres which certainly differ visibly in size. The specific size of a chromomere may well be considered as the result of the relative biochemical (catalytic) activity of the contained gene. If so, there may be a certain mass action of the gene chromatin itself which could easily be reflected in subsequent development.

Accordingly, an increase of chromosome number in polyploids attended by an increase in cell and plant size or by a slower rate of growth and regeneration, might well be the result of the added chromatin, in addition to any direct effect of the genes themselves. It is reasonably possible that the chromomere granules (or bands) are local accumulations of material synthesized by the genes and that the specific size of the chromatin granule reflects the biochemical activity of the gene.

Evidence on this very important point is still lacking but certain leads in this direction are available. It has been shown that chromosome size (and hence chromatin mass) in the larger domesticated tomato species is 30 per cent greater than that of the smaller and earlier wild form (32). The two forms presumably have approximately the same general linear arrangement of gene loci

because pairing in the  $F_1$  is fairly complete for all twelve chromosome pairs. It is possible that the increased size of the domestic form is at least partially due to its greater chromatin mass. This hypothesis would not, of course, invalidate modern genetic findings on the particulate nature of linkage relations of size genes since the chromomere chromatin itself is linearly distributed on the chromosomes in a discontinuous manner.

Whether or not a generalization could be made that the usual increased size of domesticated races is partly due to increased chromatin mass is of course very debatable. For example, there is no assurance that the domesticated tomato arose from the present wild species. But it is interesting to note that Rohweder's (43) measurements of *Dianthus* chromosomes show much the same condition

TABLE 6  
*Micromorphological data on Dianthus species in microns*  
(From Rohweder, 1934)

|                             | Chromosome<br>diameter | Chromosome<br>volume | Nuclear<br>diameter |
|-----------------------------|------------------------|----------------------|---------------------|
| 15-chromosome species       |                        |                      |                     |
| <i>Armeria</i> .....        | .28                    | .38                  | 8.0                 |
| <i>deltoides</i> .....      | .37                    | .79                  | 8.1                 |
| <i>pruinosis</i> .....      | .38                    | .83                  | 10.1                |
| <i>neglectus</i> .....      | .39                    | .93                  | 9.0                 |
| <i>alpinus</i> .....        | .44                    | 1.33                 | 8.3                 |
| <i>glacialis</i> .....      | .46                    | 1.53                 | 8.7                 |
| <i>pinifolius</i> .....     | .48                    | 1.75                 | 7.8                 |
| <i>graniticus</i> .....     | .49                    | 1.92                 | 8.8                 |
| <i>sylvestris</i> .....     | .64                    | 4.08                 | 9.0                 |
| <i>Carthusianorum</i> ..... | .73                    | 6.10                 | 9.5                 |
| <i>barbatus</i> .....       | .80                    | 8.20                 | 8.3                 |
| <i>superbus</i> .....       | .83                    | 9.00                 | 10.0                |
| 30-chromosome species       |                        |                      |                     |
| <i>Sinensis</i> .....       | .37                    | 1.6                  | 10.4                |
| <i>saxifraga</i> .....      | .41                    | 2.2                  | 9.3                 |
| <i>orbiculus</i> .....      | .53                    | 4.7                  | 9.3                 |
| <i>subacaulis</i> .....     | .55                    | 5.1                  | 9.6                 |
| <i>serotinus</i> .....      | .67                    | 9.3                  | 9.9                 |
| <i>arenarius</i> .....      | .75                    | 13.4                 | 11.3                |
| <i>Sternbergii</i> .....    | .76                    | 13.9                 | 11.5                |
| 45-chromosome species       |                        |                      |                     |
| <i>Seguieri</i> .....       | .36                    | 2.1                  | 11.6                |
| <i>caryophyllus</i> .....   | .52                    | 2.1                  | 7.0                 |
| <i>plumarius</i> .....      | .63                    | 11.8                 | 10.4                |
| <i>monspeulanus</i> .....   | .73                    | 18.4                 | 12.6                |

as may be noted in Table 6. The cultivated species, such as *D. barbatus*, *D. superbus* and *D. caryophyllus*, certainly have a significantly larger chromatin size than their wilder relatives.

In his monograph on *Narcissus*, Fernandes (19) demonstrates clearly the size differences of the chromosomes in the hybrid between *N. reflexus* and *N. bulbocodium*. In his Figure 7, it is evident that the phenotypically larger species is *N. reflexus* which also has the larger chromosomes. Chromatin size differences in the same  $F_1$  plant have also been reported in *Aesculus* (48) but not correlated with the phenotype.

While the above-mentioned evidence is not all taken from polyploid material, it does suggest that chromatin mass is directly related to phenotypic development. Accordingly, the greater chromatin mass of polyploids may well be responsible for some of the effects of increased size, but certainly not for all because in many forms neither additional chromatin size nor chromosome number has such an effect. In this case, the influence of the genes themselves outweighs that of the chromatin.

#### *Evolutionary Significance of Genes in Polyploids*

Perhaps the most important problem in evolutionary genetics is concerned with the origin of new genes. Certainly, there is no experimental evidence that genes arise *de novo*, and yet it is reasonably to be expected that highly complex forms of life have more and different genes than simple forms. To some extent, the extreme developments of domestication would also seem to call for some new genes, not necessarily in any great number, however.

Polyploidy offers a partial explanation for the emergence of new genes in a narrow sense. Whenever chromosomes are doubled, and later begin to act as bivalents instead of quadrivalents, a whole new series of loci is available for gene mutations in different directions from their former sister loci. Surely the less specialized genes could then serve as new points of departure by the mutation process.

It is a well established fact that the plant kingdom has been subjected to chromosome doubling or multiplication. The following recent table from Wanscher (50), giving the chromosome numbers of 3326 species, shows strikingly distinct modal numbers at 4, 8, 12, 14, 16, 20 and 24 chromosome pairs (Table 7). From

TABLE 7  
*Chromosome numbers in species of angiosperms*  
 (From Wanscher, 1934)

| Chromosome pairs | Monocots | Dicots | Total species |
|------------------|----------|--------|---------------|
| 3                | 5        | 2      | 7             |
| 4                | 16       | 34     | 50            |
| 5                | 8        | 28     | 36            |
| 6                | 23       | 115    | 138           |
| 7                | 143      | 290    | 433           |
| 8                | 56       | 442    | 498           |
| 9                | 43       | 155    | 198           |
| 10               | 59       | 106    | 165           |
| 11               | 26       | 201    | 227           |
| 12               | 67       | 327    | 394           |
| 13               | 13       | 75     | 88            |
| 14               | 92       | 174    | 266           |
| 15               | 8        | 46     | 54            |
| 16               | 28       | 135    | 163           |
| 17               | 15       | 81     | 96            |
| 18               | 27       | 56     | 83            |
| 19               | 5        | 45     | 50            |
| 20               | 50       | 61     | 111           |
| 21               | 48       | 31     | 79            |
| 22               | 12       | 38     | 50            |
| 23               | 2        | 13     | 15            |
| 24               | 18       | 107    | 125           |
| Total            | 764      | 2562   | 3326          |

such data as these, it is beyond any reasonable doubt that polyploidy must have been a potent factor in species development among plants. For greater details the reader may well consult the very recent review by Brink (10) on "Cytogenetic evolutionary processes in plants."

### Conclusion

In general, then, genetic evidence from polyploids harmonizes surprisingly well with concepts based on the modern gene-chromosome law of heredity. This is true for both the individual hereditary characters and the organism as a whole. With the former, it is evident that character inheritance follows the particular gene distribution even when the cytological mechanism is disturbed by the addition of chromosomes.

The organism as a whole is also influenced by polyploidy but the relations of the parts are, nevertheless, maintained. The addition

of one chromosome in a trisomic, for example, alters many individual characters and upsets the favorable balance of plus and minus factors established in the diploid by long continued selection. Nevertheless, the plant continues to function as a whole. This can mean only that there is a high degree of elasticity in an organism, affording a margin of safety for variable conditions. This may well explain the success of the mutation theory of evolution in giving new mutations time to become established and to become fitted into the germinal complex in which they arose. True polyploidy affords, in addition, extra gene loci as sources for new mutations. Such extra loci, as they mutate, must preserve a correlated function with their original sister loci and the polyploid condition would seem to afford time and protection for this process.

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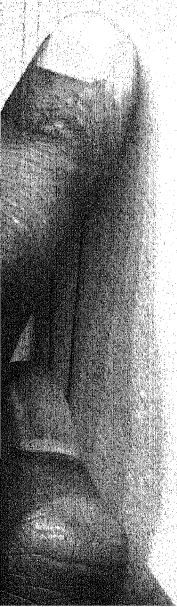
## GLOSSARY

By the editors

- allopolyploid: a polyploid possessing unidentical sets of chromosomes derived from two or more plants of dissimilar origin.—Aase.
- allosyndesis: the pairing in a polyploid of chromosomes derived from opposite parents; particularly as opposed to autosyndesis in a hybrid between allopolyploids.—Darlington.
- autosyndesis: the pairing in a polyploid of chromosomes derived from the same parent; particularly its exceptional occurrence in an allopolyploid.—Darlington.
- bivalent: during the first meiotic division, chromosomes appear singly (univalents) or in homologous groups of two (bivalents), threes (trivalents), etc.
- chromatid: a longitudinal half of a chromosome.
- chromomeres: minute subdivisions of chromatin arranged in a linear, bead-like manner on the chromosome.
- cross-over: the exchange of corresponding segments between corresponding chromatids of different chromosomes.—Darlington.
- diakinesis: the last stage in the prophase of meiosis, immediately before the disappearance of the nuclear membrane.—Darlington.
- disjunction: the separation of homologous chromosomes during meiosis.
- genotype: the kind or type of the hereditary properties of an organism.—Darlington.
- homologous chromosomes: the paternal and maternal chromatin elements which bear factors affecting the same characters.
- homozygous: possessing identical genes with respect to some character.
- meiosis: a form of cell division in which the nucleus divides twice and the chromosomes once, resulting in a reduction in the number of chromosomes.
- non-disjunction: failure of separation of paired homologous chromosomes during meiosis, resulting in their both entering the same daughter nucleus.
- phenotype: the external appearance produced by the reaction of an organism of a given genotype with a given environment.—Darlington.
- polyploid: an organism with more than two sets of homologous chromosomes; triploid ( $3n$ ), pentaploid ( $5n$ ), hexaploid ( $6n$ ), etc.
- prophase: an early stage of nuclear division.
- synapsis: pairing of homologous chromosomes during nuclear division.
- trivalent: see bivalent.
- univalent: see bivalent.



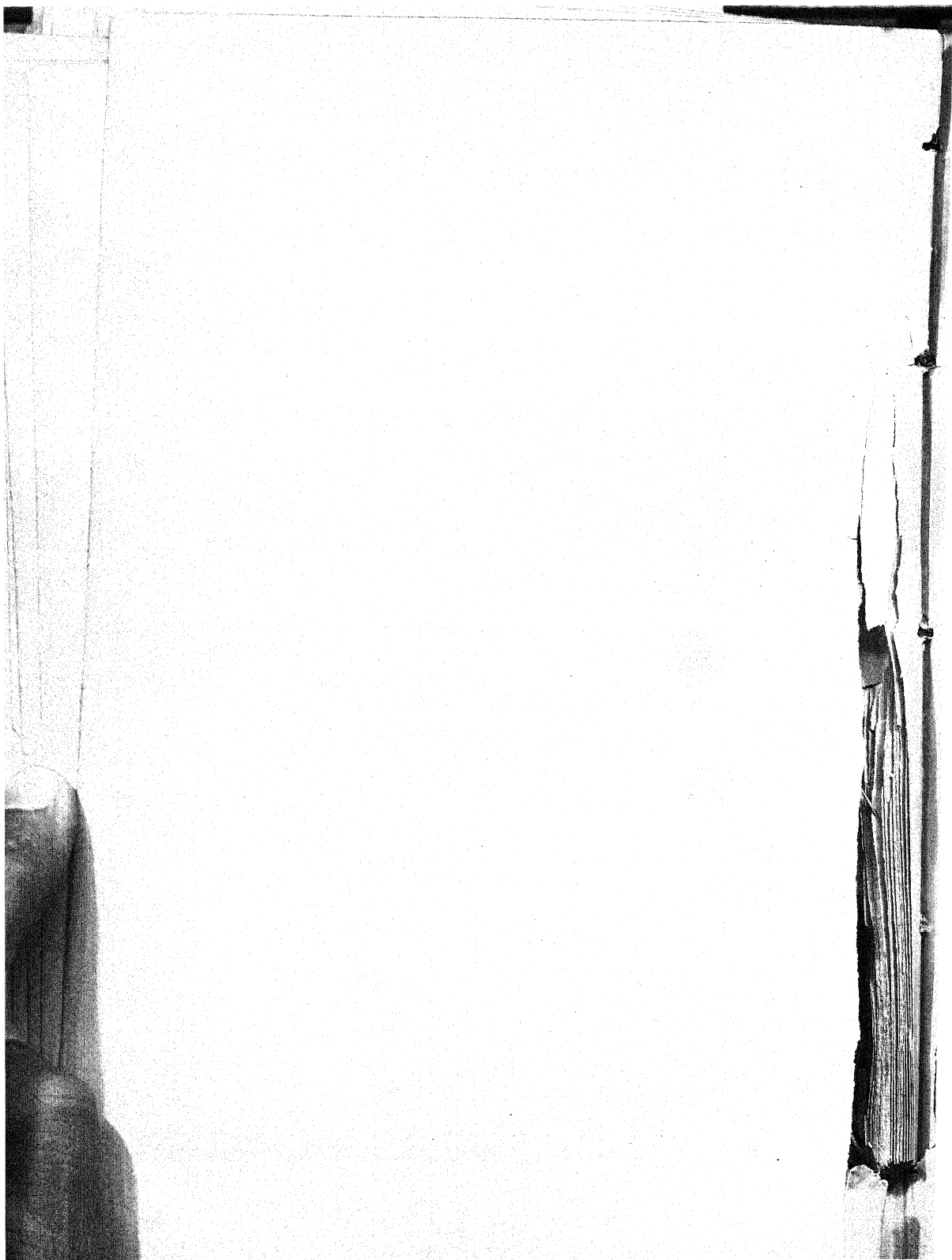
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## ERRATA

### CORRECTIONS WHICH SHOULD BE MADE IN VOLUME 1 OF 1935

- Page 18 line 2: *Rösel, von Rosenhof* should read *Rösel von Rosenhof*.
- " 18 " 28: 1924 should read 1894.
- " 62 " 19: Delete *of other films*.
- " 63 " 14: *Impossible* should read *improbable*.
- " 66 " 28: (*Fig. 3D*) should read (*Fig. 4*).
- " 68 " 1-5: Omit these lines.
- " 76 " 16: Omit (*350 x*).
- " 80 " 38: *Cardinal* should read *carinal*.
- " 110 " 34: *14-chromosome* should read *24-chromosome*.
- " 274 " 12: *or* should read *of*.
- " 274 " 36: should read *2 female, 2 male gametophytes, etc.*
- " 283 " 12: *irregularities* should read *meiotic irregularities*.
- " 287 " 24: should read *aneuploid plant has one or more incomplete sets of chromosomes*.
- " 358 " 12: *Lipmäe* should read *Lippmää*.
- " 376 " 38: *Lipmäa* should read *Lippmäa*.
- " 388 " 39: *like Bower* should read *unlike Bower*.
- " 392 " 26: *27 mm.* should read *27 cm.*
- " 393 " 20: *B. Dushiana supports* should read *B. Dushiana hardly supports*.
- " 393 " 31: *the Yeringian rocks* should read *the supposedly Yeringian rocks*.
- " 394 " 27: *Gantheliophorus* should read *Cantheliophorus*.
- " 394 " 32: *Gantheliophorus* should read *Cantheliophorus*.
- " 399 " 15: *triarch or* should read *triarch and*.
- " 399 " 24: *no leaf-scars* should read *no leaf-traces*.
- " 417 " 34: *Oppenheim's* should read *Oppenheimer's*.



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# THE BOTANICAL REVIEW

VOL. II

MAY, 1936

No. 5

## STATISTICAL ECOLOGY

ERIC ASHBY

*The University, Bristol, England*

### INTRODUCTION

It is almost inappropriate to publish an article on statistical ecology in this journal which records botanical progress, for many applications of statistics to the analysis of vegetation must be regarded as signs of decadence rather than of progress. Most biologists are not mathematically minded and the introduction of mathematics into biology is justified by one result only—a sharper mental focus of natural phenomena.

The analysis of vegetation resolves itself into two major problems, classification of plant communities and distribution of individual species within communities. The problem of classification is essentially philosophical and a correspondingly diffuse literature has accumulated, touching on such questions as whether communities are discrete units, or whether a taxonomy of vegetation is quite extrinsic to vegetation itself. This literature has already been reviewed by Pavillard in this journal (29). Some of the points he raises must be reiterated here since they require a different emphasis in their present context. The second problem, the analysis of the distribution of species, has received little attention until recently, apart from the observations inherent in the words 'abundant,' 'local,' 'rare,' familiar to field botanists. It is the purpose of this essay to examine the application of statistical methods to the specific questions arising in these two categories of plant sociology. No attempt is made to survey the literature comprehensively and these pages can serve as little more than a prelude to the original work.

### THE CLASSIFICATION OF VEGETATION

It is significant that the foundations of ecology were laid ages before the advent of ecologists. Words like 'prairie,' 'maquis,' 'moor,'

'steppe,' 'veldt' prove that the common man is capable of classifying vegetation with some precision. When these words occur in literature they summon up vivid images of the appropriate vegetation. It needs little reflection to understand that the essence of this classification is subjective, based on the physiognomy of the most prominent plants. The logic which leads a man to distinguish between a prairie and a savannah is the same logic whereby he distinguishes a dog from a jackal. In the designation of these subformations, as we might now call them, we have not progressed much farther than the founders of our language, for we find their classification adequate. Its adequacy can be illustrated by reference to the remarkable survey of African vegetation by Shantz (37), or to Livingston and Shreve's work on the climate and vegetation of the United States (23).

This subjective approach to ecology is still followed in the analysis of more local communities. The beechwoods of Europe, the mesquite of Arizona, the garigues of France, are all recognized by the practiced eye of the ecologist and assigned to their categories according to his personal judgment. The difficulties of classifying transitions were largely removed by Cowles' brilliant concept of succession (11), and although there may be disputes over individual cases, there is general agreement as to the canons of criticism to be used in plant sociology. Confusion there certainly is, but it is no worse than the parallel confusion among taxonomists over genera like *Hieracium* and *Rubus*.

As early as 1902 Jaccard (18, 19) attempted to establish more precise criteria for classifying plant communities. He compared the number of species common to two communities with the total number present in both, and expressed the result as a percentage. In the comparison of two exactly similar alpine meadows the coefficient did not exceed about 60 per cent, from which Jaccard concluded that the physiognomic criterion was deceptive, but he did not on that account relinquish the old method of classification. Moreover, the statistical method was not sufficiently sensitive, for a species which was abundant in one area and very rare in another would be classed as common to the two areas. The relative prominence of plants was left out of account.

The quadrat was introduced into ecology by Clements in 1905 (10), but it is significant that neither he nor Shantz, who used the

method extensively (36), departed from the personal estimation of communities as a basis of classification. Quadrats were used to describe the composition of communities rather than to classify them.

In 1909 Raunkiaer introduced the method of percentage frequency analysis into ecology (31). The procedure consists in throwing a quadrat repeatedly over the area to be examined and noting merely the presence or absence of the various species. Data are collected quickly and the method minimizes the personal error inevitable in the quasi-quantitative methods of Braun-Blanquet (8). From such data the community can be described in terms of the percentage frequency of its constituent species. The results are grouped in five valency classes:—0–20, 21–40, 41–60, 61–80, 81–100 per cent. It must be emphasised that Raunkiaer did not use the percentage frequency analysis as a basis of classification. He selected his units of vegetation by experience and used the quantitative data to summarize the internal composition of the community. Raunkiaer did suggest, however, that the percentage frequencies were distributed according to a 'law,' namely, that the five valency

classes stood to one another in the relation  $A > B > C \begin{smallmatrix} > \\ = \\ < \end{smallmatrix} D < E$ , i.e., in a J-shaped distribution.

In 1918 du Rietz and his collaborators published the first of a large number of papers (32, 33, 34, 35) in which the percentage frequency method was for the first time adopted as a basis for classification. It has been mentioned above that a maximum frequency occurs in the highest valency class, i.e., several species occur in almost every quadrat thrown on the community. It is this feature of Raunkiaer's work which du Rietz extended beyond its purely descriptive function and used as the basis of classification. He defined an association<sup>1</sup> as a community containing certain definite constants, i.e., species which occurred in 90–100 per cent of the quadrat-throws. The number of constant species depends, of course, on the size of the quadrat, but the relationship between quadrat area and number of constants is logarithmic rather than linear, so that a quadrat area is reached above which there is no striking increase in number of constants. This area, which cannot be determined with

<sup>1</sup> The unit of vegetation under discussion was called by du Rietz an *association* until 1927, when he changed its name to *Soziation* (35).



any precision, was defined—by a curiously circular argument—as the smallest area on which the association attains its definite number of constants!

The method was used, as Pavillard (29) has put it, to 'pulverize the vegetation' of the Scandinavian countries. The effectiveness of this process, and the consequent bewildering number of associations, is well illustrated by the following two examples. In the beechwoods of Scandinavia Lindquist (22) distinguishes thirteen associations in which *Asperula odorata* dominates: *Asperula-Anemone*, *Asperula-Galeopsis*, *Asperula-Lamium*, *Asperula-Melica*, etc. Oswald (28), in a stretch of moorland five miles by eight, recognizes 164 associations, and many 'Assoziationsflecken.'

It is unnecessary to labor the insufficiency of this attempt to classify vegetation, for the method has been abandoned by its inventors and its shortcomings have been examined elsewhere (5, 20, 27, 39). As a guide to any future attempts to classify vegetation statistically, it may be well to summarize the results of these examinations.

1: Any statistical method rests on the assumption that samples are taken at random from the population. In the method of constants the population is not sampled randomly (35), and when it is so sampled there are often no constants at all.

2: It has been shown by Kylin (20) and others on theoretical grounds, and by the present writer from field data (5), that the density (plants per unit area) is not a direct measure of the percentage frequency, but is approximately proportional to the logarithm of the frequency (*vide infra*). The 90–100 per cent frequency class embraces a very wide range of densities, sometimes a hundred times the range of the 80–90 per cent class. The maximum in the 90–100 per cent class, which is the foundation of du Rietz' method, depends, therefore, on the technique of investigation and not on any peculiarity of the vegetation. Furthermore, the plants which occur as constants are those whose area is small in comparison with the quadrat-area. Prominent plants are often excluded, so that the constants in a beechwood may turn out to be not beech trees, but *Oxalis* or *Mercurialis*.

3: This consideration introduces the third drawback of the method, the fact that its results do not always accord with common-sense observation. The community will always be recognized by its

prominent species, even if it is characterized by some obscure moss (33, 160). Implicit in the statistical analysis of the Uppsala school is a purely subjective choice of the constants. Their classification is in fact first made in the conventional manner, and the so-called statistical analysis imposed afterwards. This procedure is perfectly sound, so long as the investigator is not deluded into supposing that his classification is objective and based on the statistical analysis.

The writer's apology for describing a failure at such length is twofold. In the first place the work of the Uppsala school is often condemned without a clear idea of its weaknesses, and some of its critics have fallen into the same errors. Secondly, the writer wishes to advance the following conclusions. The classification of vegetation still rests upon personal judgment and intuition which cannot be replaced by any mechanical quantitative method. A statistical method even if mathematicians could provide such a method, would still have to be built upon a subjective primary classification. Efforts to set this aspect of plant sociology on a quantitative basis have merely confirmed the conventional methods employed by Cowles and his followers in America (11) and by Tansley (40), Moss (26), Watt (41) and others in England.

#### THE DISTRIBUTION OF SPECIES WITHIN THE COMMUNITY

Statistical methods are worthless as a basis for classification, but applied to the distribution of individual species they present remarkable possibilities. The specific problem may be stated in the following terms. If the individuals of a species are distributed randomly in a community, the sole agency which can be invoked to explain their distribution is chance. In other words, the conditions determining distribution are homogeneous over the area studied. The problems of experimental ecology arise from the fact that often the individuals of a species are not distributed at random. They are either aggregated or they give evidence of a mutual 'antagonism,' i.e., they are spaced further apart than they would be according to chance. Immediately the question is raised as to the cause of this heterogeneity. Does it lie in the microclimate, in the soil, or in the plants' relations to one another? Some technique for estimating distribution is clearly necessary. A second problem is the determination of the relative proportions of various species present in a community. The question often arises in agriculture where it is important to know

whether grazing or fertilizing a pasture leads to the increase of one species at the expense of another. Similar situations arise in academic botany, when the history of a species is followed through various stages of a succession, or when the distribution of a species is correlated with the distribution of some causal factor, such as hydrogen ion concentration. To all these problems statistical methods bring more precise definition and more certain knowledge. In the following pages the application of such methods is illustrated by reference to particular examples.

#### PREREQUISITES FOR STATISTICAL ANALYSIS

Before statistical methods can be employed at all, certain conditions have to be observed. Samples must be taken at random. The random sampling of a plant community is not always easy and the following two examples illustrate the precautions which are necessary. The first method consists in choosing some base line alongside the community (a path or a railway) and drawing on a map perpendiculars from this base line into the community, the perpendiculars being randomly distributed along the base line and of random lengths. Samples are then taken in the field from the ends of the perpendiculars. A second method is to draw a number of straight lines across a map of the community at random, and to walk along these by compass bearing, taking samples at regular intervals.

A second requirement is that data should be susceptible to analysis. Samples are usually collected from a quadrat (a rectangle is more trustworthy (9)). The data may be counts of individuals, tiller counts, estimates of dry weight or of area covered, or percentage frequencies. For the purposes of analysis counts of individuals are the most satisfactory. Where individuals cannot be distinguished, some morphological character, like tiller count, is suitable. If this is impracticable, estimates of percentage area covered have to be made but the data so obtained are of less value because they cannot be treated by simple statistical analysis (*vide infra*). Finally, the data are often collected as percentage frequencies. These data are still more awkward to handle. They bear no predictable relation to the actual density, unless the distribution is random, and it would be precarious to draw any conclusions as to the internal composition of a community from percentage frequencies alone.

## TESTS FOR RANDOM DISTRIBUTION

Where it is possible to count individuals, the data collected from several quadrat throws may be classified into the number of throws in which no individuals occurred, the number in which one occurred, the number in which two, three, four, occurred, and so on. The quadrat size is adjusted so that the great majority of the quadrats are empty. Under these circumstances the chances of finding 0, 1, 2, 3, 4, . . . x individuals per quadrat is given by the successive terms of a Poisson series:—

$$e^{-m}, me^{-m}, m^2/2! e^{-m}, m^3/3! e^{-m} \dots m^x/x! e^{-m}$$

where  $m$  is the mean frequency of occurrence in the samples, i.e., the total number of individuals counted divided by the number of quadrats. The procedure in testing for homogeneity is to compare the observed and calculated frequencies by means of the  $X^2$  test, in the manner described by Fisher (12). An example of the operation of this method is given in the following table:—

TABLE I

| Number of individuals | Number of quadrat throws:— |            |
|-----------------------|----------------------------|------------|
|                       | Observed                   | Calculated |
| 0 .....               | 268                        | 264.9      |
| 1 .....               | 262                        | 264.9      |
| 2 .....               | 136                        | 132.4      |
| 3 .....               | 43                         | 44.1       |
| 4 .....               | 11                         | 11.0       |
| 5 .....               | 0                          | 2.2        |
| 6 .....               | 0                          | 0.4        |
| Total .....           | 720                        | 719.9      |

The value of  $m$  is .97;  $e^{-m}$  is, therefore, .379. The column of calculated values is obtained by working out the terms of the Poisson series, and multiplying each by 720. An example of the application of this technique is to be found in Blackman's work on pastures (7). Blackman's conclusions from observations on a great number of pasture plants are that the common species are distributed at random, and the rarer species are aggregated, i.e., the distribution of dominants accords with the expectation from Poisson distribution,

while that of the occasional species does not. If it is once established that a species is randomly distributed, the number of individuals in any homogeneous area can be calculated simply from knowledge of the number of quadrat throws in which the species does *not* occur, for the zero class of the Poisson distribution,  $e^{-m}$ , determines the rest of the distribution.

Such a comparison is excellent as a test of homogeneity, but it gives no measure of the degree of non-randomness of a heterogeneous distribution. A method has recently been published which measures the degree of aggregation or of under-dispersion in a population of individuals (5). The experimental procedure involves sampling the population with a quadrat divided up into a lattice of 16 or 25 equal squares. The observations taken are (1) the total number of individuals in the quadrat, and (2) the number of empty squares. In a lattice of 25 squares, if there are no plants, there will be 25 empty squares. Above a certain density all the squares will contain at least one plant,—there will be no empty squares. Between these extremes there will be a relation between the density and the number of empty squares; this relationship is given by the equation

$$E = n \left( \frac{n-1}{n} \right)^s$$

where  $E$  is the number of empty squares,  $n$  the total number in the lattice (25 in this case) and  $s$  the density, i.e., the number of individuals in the whole quadrat. Now if individuals are clumped together, there will be more empty squares for a given density than would be expected by chance. If the individuals are mutually antagonistic the observed number of empty squares in the quadrat will be less than the calculated number. Departure from a random distribution is tested by comparing the difference:

Sum of  $E_{cal.}$  and Sum of  $E_{observed}$

with the variance of the observations. If departure from randomness is significant it can be estimated by applying the following correction to the equation:

$$E_{corrected} = n \left( \frac{n-1}{n} \right)^s \left\{ 1 + s(s-1)c \right\}$$

The value of  $c$  is a measure of the heterogeneity, i.e., it measures the degree of aggregation among clumped species or the degree of

antagonism between dispersed species. For an example of the operation of this method the reader is referred to the original work (5). The data obtained are useful in that they indicate where the problem lies. Aggregation is often found, for instance, where there is no obvious reason why the individuals should be clumped together; and in cases where competition occurs between individuals, the degree of competition may be measured.

Very recently (9a), Clapham has published a statistical analysis of some data of Steiger (*Ecology*, 11, 1930) on the distribution of prairie plants. He estimates the degree of departure from a Poisson distribution, i.e., the degree of non-randomness, by the relative variance:—

$$\frac{(x-m)^2}{m(n-1)}$$

where  $(x-m)$  is the deviation of an observation  $x$  from the mean,  $m$ , and  $n$  is the number of observations. This expression is summed over the observations. In a random distribution the relative variance is equal to unity, and it is greater than unity if there is aggregation. Its magnitude is a measure of the amount of aggregation. This method has an advantage over that suggested by the present writer (5), namely, that it is more convenient to use and less laborious to work out. As yet, no comparison has been made of the estimates given by the two methods. Using his method Clapham shows that most of the species listed by Steiger show aggregation. He points out that as a consequence of the over-dispersion of species the percentage frequency cannot be used for estimates of density, nor is an estimate of mean density itself of much value to the ecologist.

#### THE MANIPULATION OF DATA ON TILLER COUNTS

If the tillers of a species are normally distributed the frequencies will lie on a binomial distribution. Blackman has plotted the distributions for various pasture grasses in England, and finds that the dominant grasses in a sward are in fact normally distributed, while the distributions of the less common grasses are markedly skew. The causes of this departure from normality have not received experimental investigation.

#### MEASUREMENTS OF PERCENTAGE AREA COVERED

Where the counting of individuals or tillers is not feasible, the investigator must fall back on estimates of the percentage of area

covered (Deckungsgrad). Such estimates serve well for the examination of gross changes in the composition of a community (*vide infra*) but they are unsuitable as a means of testing homogeneity. The reason is obvious. A test of homogeneity must be made on discrete units of the same size. Any statistical test of units which themselves vary in area (e.g., patches of clover in a lawn) is invalid. Both Blackman (7) and Hanson (17) have published distribution curves of percentage area covered. The curves are extremely skew, so much so that the testing of homogeneity from replicate plots by analysis of variance, as Hanson has done (17), is probably not admissible. The analysis of variance is becoming increasingly useful as an instrument for the analysis of biological data. It is important to remember, therefore, that its value decreases as the distributions to which it is applied depart further from normality. To this particular instance of area distributions, as Blackman has pointed out, the analysis cannot be legitimately applied unless some sort of transformation is carried out on the original data, to restore them to normality.

#### PERCENTAGE FREQUENCY DATA

Observations on species distribution which consist solely of percentage frequency measurements can provide no information as to homogeneity. At a previous point in this essay the inherent ambiguity of the method was mentioned. This ambiguity lies in the non-linear relation between density and percentage frequency. The relation in a homogeneous population is given by the equation

$$P = 1 - e^{-kx}$$

where  $P$  is the frequency,  $x$  the density, and  $k$  the size of the quadrat. Now, although there is no direct proportionality between  $P$  and  $x$  over their whole ranges, values of  $P$  are approximately proportional to  $x$  over a narrow range of frequencies. By an adjustment of the quadrat size so that most of the densities fall into the 80 per cent frequency class or lower, and none in the 100 per cent class, the percentage frequency becomes an approximate measure of the density. A mathematical demonstration of this principle is to be found in the appendix to Blackman's paper (7; 775). It should be emphasized that as an estimate of density this technique is a last resort, for it depends on the assumption of homogeneity, an assumption which is often unjustified.



THE USE OF STATISTICS IN FOLLOWING CHANGES IN RELATIVE  
COMPOSITION

The importance of being able to follow changes in the relative composition of pastures has led to several statistical methods of pasture analysis, which differ rather in their experimental technique than in their logical basis. The percentage frequency method may be set aside as useless, except in the modified form suggested above. For example, if measurements are made with a 100 cm<sup>2</sup> quadrat, a change from 2 plants per quadrat to 16 per quadrat would involve *no* change in percentage frequency, while a change from 2 plants per quadrat to one plant would be recorded as a change of 50 per cent!

Changes in botanical composition may be followed easily by tiller counts, or measurements of percentage area covered. The technique applied to the analysis of manurial effects on lawns is well illustrated by the following table from Blackman (7); the figures represent percentage areas covered by three constituents; two grasses and the total "weeds," under two treatments and the control.

TABLE II  
PERCENTAGE AREA COVERED

|                           | Control |         | Sulphate of ammonia |         | Ferrous amm. sulphate |         |
|---------------------------|---------|---------|---------------------|---------|-----------------------|---------|
|                           | June    | October | June                | October | June                  | October |
| <i>Poa</i> spp. ....      | 13.3    | 22.0    | 34.1                | 38.0    | 38.3                  | 58.8    |
| <i>Agrostis</i> spp. .... | 31.5    | 36.9    | 53.2                | 58.0    | 48.4                  | 41.4    |
| Total "weeds" ...         | 36.2    | 33.8    | 11.7                | 5.0     | 12.5                  | 0.3     |

The differential effect of fertilizers in this instance is evident without further analysis. The same technique has been applied by the writer to an examination of the change in abundance of *Galium saxatile* and other species at the transition between one association and another, on the slope of a mountain in Wales. It is evident from the results that the transition between the two associations is abrupt, not only for the dominant species, but for many of the subsidiary species too. The cause of this abruptness lies probably in the influence of the dominant species upon the local environment of the



less pretentious plants in the association, and it cannot be invoked as evidence of the individuality of associations (*cf.* 33).

In Australia changes in relative composition have been followed by Levy and others (21) by the "point" method. Hanson (17) has compared this with other methods of collecting data on botanical composition and finds a general agreement in results. The mode of dealing with the data is the same.

#### THE CORRELATIONS OF SPECIES DISTRIBUTION WITH ENVIRONMENTAL FACTORS

Statistical methods are appropriate for investigating the causal relation between plant distribution and some environmental factor. In such an enquiry it is necessary first to unravel the distribution of the species in question from the distribution of the factor under observation, and subsequently to seek some correlation between them. It has been alleged, for instance, that there are in Nature definite pH optima for some species. It is easy to understand how this opinion arose. *Pteris*, for instance, is found in England on soils of pH 5.6 more commonly than at any other pH. Statistical analysis showed, however, that the cause of this is *not* a peculiar preference of *Pteris* for that particular pH, but is merely the circumstance that a pH of 5.6 is more frequent than any other pH on these particular sandy soils. The distribution of telegraph poles on these soils would also show an optimum pH at 5.6. The method of analysis employed in this instance is extremely simple. Frequencies of *Pteris* were expressed as percentages of the frequencies of pH. These percentage occurrences of *Pteris* showed no preference for any particular pH, i.e., within the range at which *Pteris* will grow at all, one pH is as good as another. The comparison of observed and calculated percentages was made by means of the  $X^2$  test (13).

By a similar procedure the abundance of one species may be correlated with that of another. A significant correlation may indicate competitive effects. Often two species are surprisingly independent of one another in their distribution, i.e., they seem to co-exist in the same quadrat without getting in each other's way. Thus Hanson (17) calculated the correlation between the tiller counts in the same quadrat of *Bouteloua* and *Agropyron*, and obtained a value for the correlation coefficient of  $.09 \pm .034$ , which is insignificant.

## SPECIES AND AREA

Several attempts have been made to derive a formula for calculating the probable number of species in an area from observations of the number of species in small samples of the area. Arrhenius (1, 2, 3) suggested a formula which he illustrated by data of his own and which was subsequently applied to data of Gleason (14, 15) and du Rietz (34). Gleason showed that the formula gave results far in excess of the observed values, when applied to large areas, and du Rietz gave evidence that the agreement failed at areas as low as 4 square metres. The premise of Arrhenius' formula is unsound, for the expression is parabolic in form, and, consequently, with increasing area the number of species increases to infinity! Gleason put forward another formula which has the merit of being theoretically sound, for it is logarithmic, and hence is asymptotic along the area axis, i.e., it does not presuppose an infinite number of species. If it holds over large areas it does presuppose, however, equality of environmental conditions, and Gleason has used the successful application of his formula as evidence of such homogeneity.

The requisite procedure in order to verify Species-Area equations in general is to take readings of the number of species in quadrats of different areas laid at random over the community. It is not legitimate to 'compose' the readings for larger quadrats by combining the data from the small ones, for this treatment cancels out any inequalities in distributions. Since most of the data purporting to verify these equations have been collected in this erroneous way, it is premature to discuss the value of the equations themselves.

## CONCLUSIONS

A study of the literature to which this essay is an introduction will convince the student that statistics have been applied to the most varied ecological problems, often needlessly, and sometimes on false assumptions. It is a literature remarkable for its prolixity, but from its pages there emerge methods which are new to biology and which prove valuable in the difficult study of plant distribution. It must be left to the future to decide in what measure these methods can advance botany.

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## THE PHYSIOLOGY OF HOST-PARASITE RELATIONS

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### INTRODUCTION

In reviewing the subject of parasitism from the physiological standpoint, little useful purpose would be served by confining attention to the literature of the last few years. References to the physiological relations of host and parasite are often of a casual nature, made in the course of investigations which had some other object in view, and even if one succeeded in collecting and evaluating these rather miscellaneous observations or interpretations, no satisfactory picture of the problem would emerge. It is proposed, therefore, more especially as this is the first of these reviews which deals with the physiology of parasitism, to make a general survey of the literature, in the light of which the more recent developments will be more readily understood.

The earliest substantial contribution to the subject is by De Bary (6) in a classical paper dealing with the mode of invasion of *Sclerotinia libertiana*, a non-specialized type of parasite. Examination of infected tissue (broad bean, carrot, etc.) showed that two types of action had occurred; one, the solution or partial solution of certain constituents of the cell walls whereby the tissues lost coherence or in other words were rotted; and the other, the killing of the protoplasts themselves. De Bary further demonstrated the presence, in extracts of tissues parasitized by the fungus, of an active principle which produced the effects described. Boiling of these extracts in general destroyed their activity, whence the conclusion was drawn that the effect on the cell wall was due to an enzyme. The nature of the toxic or killing substance was less clear and De Bary, in effect, left that question open. In this connection he considered oxalic acid, which is known to be produced by a number of fungi, but the evidence that this acid was produced in sufficient concentration by *Sclerotinia libertiana* was unsatisfactory.

In studying the earliest phase of attack, i.e., penetration of the intact surface of the host, De Bary reached certain conclusions which

have markedly influenced subsequent workers in this field. By a variety of experiments he showed that the hyphae of *Sclerotinia*, when placed on a suitable plant part, attacked only after they had been given a certain amount of feeding, whence his conclusion that parasites of this type achieve parasitism by way of an intermediate stage of saprophytism. When the hyphae were placed in a nutrient medium on the intact surface of a susceptible host, De Bary claimed that they penetrated directly, whereas when placed in water they were unable to do so at first. Instead, they applied themselves to the host surface and formed attachment organs ("appressoria"). From these a toxic principle was excreted into the underlying living cells which were thereby killed. The soluble contents of the dead cells now diffused out to the surface where they supplied the saprophytic feeding necessary for the establishment of parasitism.

It is important to appreciate this point because, if true, it would indicate a possible difference between a parasitic and a saprophytic fungus, the former having the capacity of killing the underlying host cells before penetration had taken place, and the latter presumably being unable to do so. Incidentally, it may be noted that De Bary was unable to explain satisfactorily this "killing in advance of penetration" on the basis of any of the extracts prepared by him. When these were laid on the epidermis of a plant, action was very uncertain and in many cases there was none. He suggested, rather weakly, that the killing principle was deactivated before it had time to diffuse through the cuticle.

While, as will be shown later, there are grounds for not accepting the thesis of killing in advance of penetration, there is general agreement that, once a fungus of the type under consideration has entered the tissues of its host, there is a more or less well defined killing and maceration of the host cells in advance of the region occupied by the hyphae.

De Bary's work was in the main confirmed and in certain respects amplified by Marshall Ward's (138) study of a lily disease caused by a species of *Botrytis*, and by Nordhausen (106) who examined the behaviour of a number of fungi, more particularly that of *Botrytis cinerea*, a widely distributed parasite. Similar conclusions were reached by some of the earlier students of bacterial plant diseases, e.g., by Jones (81) and Van Hall (135) for strains of *Bacillus carotovorus*. On the other hand, Behrens (7) claimed that the toxic

principle of certain fruit-rotting fungi was a thermostable substance, and an extreme position was taken up by Smith (124) when he stated that both the killing and macerating effects of *Botrytis cinerea* on lettuce tissue are brought about by oxalic acid secreted by the fungus.

A study of the earlier papers cited above shows that in no case was the fungal extract employed of reasonable activity. The experimental method was to immerse pieces of sensitive tissue (turnip, potato, etc.) in the extract and to note the changes brought about. Immersion in itself is injurious to the tissue if it is long continued, and to that extent the action of any active principle concerned is obscured. To judge from the records of earlier experiments, the reaction times were unduly long—often from one to several days. In so far as these experiments had reference to a toxic principle, an antiseptic could not be added to the preparations, as is usual in enzymic work, so that there was the further difficulty arising from bacterial contamination. Again, some at least of the preparations used were obtained from old cultures of the organism, e.g., in one of Behrens' experiments from a three-month-old culture of *Penicillium* sp. on apple fruit, so that there is the double uncertainty as to how far the active principle of the fungus was contaminated with host residues and with fungal products which are associated with old and degenerate mycelium.

With these considerations in mind, Brown (14) prepared an extract from the young germ tubes of *Botrytis cinerea* and, as this proved to be of high activity, it was very suitable for the study in question. This extract actively decomposed a great variety of tissues, such as vegetable and fruit tissues generally, floral parts, the leaves of many plants and the stems or leaves of succulents. Lignified parts were, as far as could be seen, unaffected and so also were the delicate tissues of mosses and liverworts. The action was of the twofold nature outlined by De Bary. Heating for a short time to 60–70° C. destroyed the activity of the extract as regards both killing and macerating effects. Attempts were made in various ways—by deactivation of the enzyme by heat, by mechanical agitation, by the addition of a suitable small quantity of alkali, by precipitation with alcohol, and by fractional dialysis with a series of graded membranes—to separate an enzymic from a toxic principle but with no success. Though, as was pointed out

at the time (20), none of these experiments was definitely critical on the point, the obvious interpretation of the results was that both activities concerned were due to one and the same substance, a cytolytic enzyme ("pectinase," in the older literature "cytase"). Certainly there was no thermostable crystalloidal toxin present in the extract of *Botrytis* germ tubes, and oxalic acid in particular, as the killing agent, was further excluded by the fact that the extract contained a trace of a soluble calcium salt.

Similar active preparations, though not so pure as the one just described, were obtained (16) by using the liquid in which a dense suspension of *Botrytis* spores is terminated. The active principle is thus freely excreted into the nutrient medium. Under a given set of cultural conditions, it reaches a maximum concentration in the medium, but being somewhat unstable in solution it gradually disappears.

While the preparations so obtained fully explained the action of *Botrytis cinerea* on susceptible tissues, they did not explain the initial entry of the parasite (15). Even the strongest extracts were, with rare exceptions, quite innocuous when laid on the intact surface of susceptible plant parts. The cuticle was not attacked and the underlying tissue was not disorganized. The conclusion, therefore, was either that an important principle had not been extracted or that the "killing in advance of penetration" claimed by De Bary was unfounded, at least for *Botrytis cinerea*. Cytological investigation was taken up at this point when it was shown by Blackman and Welsford (9) for *Botrytis cinerea*, by Boyle (11) for *Sclerotinia libertiana* and by Dey (33) for *Colletotrichum lindemuthianum* that the process of penetration preceded killing of the underlying host cells. Once the cuticular barrier had been passed, killing and macerating effects quickly followed. By use of an electrical conductivity method, Brown (17) also showed that the rapid exosmosis of solutes from the tissues does not take place until some of the fungal hyphae have penetrated. The effect of these investigations was, therefore, to cast doubt upon De Bary's view according to which parasitic fungi of the facultative type are able to attack the cells of the host plant prior to penetration of the cuticle.

#### THE STAGES OF PARASITISM

In the light of the general picture outlined above, a review of the literature bearing on the physiology of parasitism will now be set



out, and it will aid to a methodical treatment if the subject is subdivided according to the stages which can be distinguished in the process of establishing parasitism, viz., the stage before penetration, penetration itself, and the stage after penetration. The discussion under the first two headings will be general, as there is no evidence that the behavior of one type of fungus is essentially different from that of another. When the third stage is reached, a further subdivision will be necessary as important differences then arise as between facultative and obligate parasites. Throughout the discussion it will be assumed that the parasite concerned is a fungus; with slight modifications here and there, the same considerations apply to bacterial parasites.

*Stage before Penetration.* The factors which come up for discussion are those which influence the germination and growth of the parasite. Fungal spores have a capacity for germinating in pure water which depends upon the species, the age and pre-treatment of the spores. Some are able to germinate vigorously on their own reserves and are to that extent more capable of parasitising than others which require an extraneous source of food for the purpose, i.e., the "saprophytic nourishment" postulated by De Bary. Under natural conditions suitable for infection, the spores are, however, not always, or perhaps even often, in contact with pure water on the surface of a plant. By laying drops of distilled water on the intact surface of a variety of plant parts, Brown (17) showed that a passive exosmosis of electrolytes took place from the tissue into the drops, and that in many cases the solutes which so diffused out markedly stimulated the germination of spores. With other tissues the effect was the opposite, i.e., there was more germination in distilled water than in drops which had lain for some time on the surface of the plants. It was further shown (18) that volatile substances given off by plant tissue had a like effect upon germination, so that under conditions where these substances could accumulate in the atmosphere the parasitic vigor of any fungal spores present would be influenced.

The effect of an extraneous source of food on the initiation of disease may be illustrated by a few examples. Attack on foliage leaves may result from their contact with moribund floral parts. This is a fact well known to tulip growers. Similarly, the *Botrytis* disease of lettuce seedlings (1) generally begins by the attack of the

withered cotyledons or lower leaves. The spur-blight disease of certain fruit trees (147) has its beginning in the germination of the fungal spores on the nutritive stigmatic surface of the flowers. The dependence of the sooty-mold type of disease upon the presence of honeydew illustrates the same point, though in this case true parasitism, in the sense of invasion of the host tissue, does not as a rule occur.

The pathogenicity of soil-borne fungi is influenced in a similar way. Thus it is a very general experience that sterilization of a soil by heat for the control of a soil-borne disease is liable to accentuate the trouble, unless the operation is carried out so effectively as to eliminate the parasite completely. Similarly, workers on the foot-rot diseases of cereals all agree in stating that infection takes place with much greater readiness in sterilized than in unsterilized soils. The enrichment of the soil solution which follows from the heating process would partly explain these results but there is also considerable evidence that microorganisms in competition interfere with each other's activities, partly, no doubt, by using up the available food and partly by a deleterious action of their metabolic products. The literature of this so-called "biological antagonism," in so far as it concerns the cereal foot-rot fungi, has been recently reviewed by Garrett (47).

In addition to nourishment, two other factors which determine the behavior of the fungus in the stage preceding penetration are temperature and moisture. Plant pathological literature contains a vast number of references to the working of these factors.<sup>1</sup> For the present review it is sufficient to state that, for the establishment of parasitism, temperature must not range beyond certain limits which are more or less definite for each disease, and that moisture must be present, either as free water, or as water vapor at a concentration not too far removed from the saturation point. Sufficiently humid conditions must also be maintained for a time long enough to enable the spores to germinate and the germ-tubes to be established within the host tissue, whereby the parasite becomes less liable to check by desiccation. Hence the importance of continued rainy or "muggy" weather (as with *Phytophthora infestans* on potato) and of long dew periods (as with rusts and mildews).

<sup>1</sup> No attempt will be made in this article to review the very extensive literature which deals with the effect of environment upon the incidence of disease.

The conditions of wetness necessary for the initiation of attack may be difficult to attain on account of certain structural arrangements of the plant. Thus the surface of some plants is covered with a waxy bloom, which is more pronounced in some environments than in others, or with a dense layer of hairs, so that the surface is very difficult to wet. Drops of water which may contain the spores of a parasite readily fall off, and so the mechanism of parasitism breaks down at the very beginning. It is customary to speak of such cases under the heading of "disease escape" inasmuch as the plants may be readily attacked if the fungus is only allowed to establish contact. This distinction seems hardly logical, for such plants have in fact a structural device which defeats the potential parasite at the outset, and their resistance is no less real in that it came into play at the beginning rather than later on.

✓ *Phase of Penetration.* Three ways of entry are possible; directly through the epidermis, through stomata or lenticels or water pores, or through wounds. The last may arise in the normal course of the plant's growth, e.g., at leaf-scars or at the points of emergence of lateral roots, or they may be caused by external agents, such as wind, insects, etc. Whatever mode of entry is adopted, the question arises as to whether the fungus enters by accident or whether it is attracted by some stimulus on the part of the host. When entrance takes place through the epidermis there is the further question as to how the resistance of the epidermal wall and, in particular, of the outermost cuticularized layer is overcome.

The view that the stimulus which leads to penetration is of a chemical nature was the natural outcome of Pfeffer's (109) researches on the locomotory directive movements of motile cells (zoospores, antherozoids) and was developed by Miyoshi (98, 99) working in Pfeffer's laboratory. According to this view, various substances such as sugars and salts diffuse out from the living cells of the plant and through the outer epidermal wall. Fungal hyphae which may be present on the surface react to this unilateral stimulus by turning in such a way as to grow up the gradient of attracting substance or, in other words, towards the source of the latter which is the host tissue.

Miyoshi's experimental methods, which have been largely followed by later workers, were briefly as follows. A solution of the substance under test was placed in a glass capillary which was then

laid in a drop of water containing germinating spores. More frequently the solution, solidified with agar or gelatine, was placed on one side of a permeable membrane (collodion, natural epidermis, etc.) or of a plate of impermeable material, such as mica, which was provided with a number of small holes. The spores were placed in a film of plain agar or gelatine on the opposite side. Conclusions were drawn according as the germ tubes grew towards or away from the source of the chemical substance or behaved neutrally. From experiments of the kind indicated Miyoshi constructed an elaborate theory of chemotropic attraction by certain substances and by plant extracts. Some substances were attractive, others not, and the concentration of an attractive substance must, on the one hand, exceed a certain threshold value, otherwise there would be no response, and, on the other hand, must not exceed a certain maximum, otherwise the effect would be one of repulsion. Penetration through membranes did or did not take place according as a suitable concentration of an attractive substance was or was not present on the opposite side. There are some difficulties and inconsistencies on certain points in Miyoshi's papers, but on the whole the evidence is both circumstantial and convincing.

Unfortunately for the theory of chemotropism, Miyoshi's conclusions were to a very large extent called in question by the later work of Fulton (46). Working by the same methods and with very much the same kind of fungi, he failed to obtain any such marked tropisms as had been recorded, the fungal hyphae showing on the whole as much turning towards pure water as to a solution of the presumably attractive substance. On the contrary, Fulton claimed that the only marked tropism shown was a turning away from the metabolic products produced by the fungus itself, and he, therefore, substituted for the positive chemotropism of Miyoshi a negative tropism of the kind stated. A similar conclusion, though founded on less extensive experimental work, had been reached a few years previously by Clark (26).

The subject was again taken up by Graves (58) who used the mica-plate method and adopted a hard-and-fast system in the ascertainment of his data whereby any liability to personal bias would be eliminated. His general conclusion was that there was justification for both Miyoshi's and Fulton's views, but that the negative chemotropism of the latter was the greater effect. Graves'

paper is the last of any importance on the subject, and though certain points are still rather obscure it must be accepted as the best account available for the time being.

Granted now that the two kinds of chemotropism are well established, it remains to consider how far the one or the other can be applied to explain the entrance of a fungus into its host. On this point very diverse views have been expressed. At the one extreme, Massee (94) founds a theory of parasitism upon the lines of Miyoshi's work. Susceptible plants are such by virtue of possessing in their cells certain attractive substances, and conversely resistant plants are devoid of these. A specialized parasite is one which responds to a particular substance only, whereas a generalized parasite responds to many substances. These views are very far from the truth if only for the reason that fungal hyphae have repeatedly been observed to enter the tissues of plants which they are unable to parasitize. At the other extreme, Brown (21) has argued that neither of the tropisms referred to plays any significant part in the process of penetration.

As far as the entrance of germ tubes through stomata is concerned, none of the experimental evidence alluded to is relevant. The perforated mica-plate, which has been so much used in work on chemotropism, is presumed to be a working model of an epidermis with its stomata. But there is an important difference, viz., that, whereas there is diffusive continuity through the holes of the mica-plate so that water-soluble substances can pass from one side to the other, the substomatal cavity is in general filled with air. If, therefore, there is any tropism to substances coming out through stomata, these substances must be volatile, and none of the investigations cited has furnished evidence of a positive tropism to gases. The only work which is significant on this point is that of Balls (4) who states that germ tubes of rust spores grow through holes in a rubber membrane when an atmosphere saturated with water vapor is maintained on the opposite side. Hence a tropism towards water vapor is suggested.

According to Arens (3), the zoospores of *Plasmopora viticola* tend to congregate around stomata, especially when these are open, and they behave in this way towards the stomata of many plants other than vine. The stimulus in this case cannot be water-vapor since the zoospores are swimming in water. ✓

In considering the penetration of stomata by germ-tubes one probably should distinguish two phases, first the growth of the germ-tube up to the stoma, and second the entrance of the germ-tube into the substomatal chamber. It does not seem necessary to postulate any directive stimulus for the first of these stages, for no difficulty is involved in assuming that a germ-tube grows accidentally over one stoma or another. The second stage of the process would hardly be accidental, at least with the germ-tubes of rust summer-spores. When these arrive above a stoma they very characteristically form a swelling, thus indicating some influence emanating from the stoma, and subsequent growth is directly downwards into the sub-stomatal chamber.

Coming now to penetration through the epidermis, one might suggest that the negative chemotropism of Fulton and Graves causes the germ-tubes to grow away from a region of high concentration of fungal products, viz., the water drop in which the spores have germinated, into a region of low concentration, viz., the underlying host cells. But this argument is fallacious. The germ-tubes can respond only to differences of concentration within the infection drop, and it is more rational to suggest that the highest concentration of metabolites would arise on the sides of the germ tubes next to the epidermis, where free diffusion is hampered by the proximity of the cuticle. The result would, therefore, be that the germ-tubes would turn away from the surface of the plant.

The positive tropism of Miyoshi and Graves is, however, not ruled out so simply. It is quite conceivable that germ-tubes growing close to the epidermis may react to a one-sided stimulation of substances diffusing outwards from the tissue. On the other hand, Brown and Harvey's (19) work with spores of *Botrytis cinerea* and epidermis of *Allium cepa* showed that penetration took place freely after the strips of epidermis had been subjected to prolonged washing—which should have removed all traces of diffusible substances—and, furthermore, that the hyphae grew with equal freedom from the inner surface to the outer, i.e., with or against a possible gradient of chemotropic substance. In experiments with membranes of formalized gelatine the hyphae penetrated independently of the initial distribution of the food substance. Finally the fact that membranes of paraffin wax were readily penetrated (as had also been shown by Miyoshi) proved that a chemotropic stimulus is not necessary for penetration.

The lack of evidence in favor of chemotropic theories drives one back upon a theory which has been suggested from time to time, viz., that the stimulus to penetration is a contact one (haptotropic). While this theory has the disadvantage of not being easily explored experimentally, there are no fundamental objections to it, and all the evidence available is in its favor. The germ-tubes of fungi do react to contact, as is well shown with *Botrytis cinerea*. Contact with a hard substance induces a number of changes in the germ-tubes: the tips become rounded and somewhat swollen, growth in length is temporarily stopped, and the enlarged apex becomes firmly attached to the solid object, forming an "attachment organ." From the area of contact and at a short distance behind the extreme tip of the hypha, a fine penetration tube is put out through the underlying surface, if this should happen to be penetrable. The same sequence of appearances is seen when the spores are germinating on the surface of glass, except that of course no penetration is possible. One may, therefore, plausibly suggest that the same stimulus is operating in both cases, viz., the contact with a hard surface. Whether the physical character of the hard surface, as for example its roughness, influences in any way the nature of the response called forth is quite unknown.

With fungi which penetrate directly through the epidermis of the host the further problem arises of the mechanism of this process. Here again two theories have been advanced, a chemical and a mechanical. The general tendency has been to postulate a chemical action upon the cuticle, but the positive evidence in favor of this is not strong. As has been pointed out, Brown failed to discover any evidence of a cuticle-dissolving enzyme in the most active extracts of *Botrytis cinerea*. Thus an extract which on injection completely destroyed rose petals within a quarter of an hour produced no effect whatever when laid on the surface of petals for 24 hours, whereas spores so placed had disorganized the tissue in some 8 to 12 hours. The parallel cytological investigations agreed in indicating no solvent effect of the fungus upon the cuticle.

A somewhat different view is expressed by Wiltshire (146) in his study of the apple-scab organism. He considered that a cuticle-dissolving enzyme was present inasmuch as the cuticle became thinner when it was sloughed off by the development of the fungus within the tissue. But this does not mean that the outermost

cuticular layer, which is presumably most heavily impregnated with wax-like substances and which is the only layer significant to the process of penetration, is affected. Perhaps one of the most categorical claims in this connection is that contained in a recent paper by Chaudhuri (23). According to this paper the cuticle of *Citrus* sp. is attacked by an exudation from spores of the "wither-tip" fungus with the result that it is made easily penetrable by the fungal hyphae. The test applied is the capacity of the cuticle to take up a certain dye, but this in the writer's opinion gives no grounds whatever for drawing any conclusions relative to the penetrability of the cuticle by fungi.

The ability to penetrate membranes in a purely mechanical manner has been definitely proved for some fungi. Miyoshi demonstrated the penetration of collodion and gold-leaf films, and the writer has used membranes of paraffin wax for the same purpose. With a series of formalized gelatine membranes of graded hardness it was found that the germ-tubes of a particular fungus penetrated membranes up to a certain grade of hardness, and that beyond this point long-continued exposure did not lead to penetration. Chemical solution of the membranes is thus ruled out and the mechanical theory is the obvious one. Similarly in experiments on the penetration of *Eucharis* epidermis, Brown and Harvey (19) found that penetration took place if the leaf tissue was plasmolyzed so that the hydrostatic backing to the cuticle was removed, whereas the epidermis of a turgid leaf was not penetrated. This result can readily be interpreted on the mechanical theory but it is very difficult to understand why, if the fungus produces a cuticle-dissolving enzyme when growing on the flaccid epidermis, it should not be able to do so when the epidermis is rigid.

A necessary condition for mechanical penetration is that the fungus should be anchored to the epidermis of the host, i.e., there must be an appressorium. The thrust on the epidermis is reduced to a minimum by the fact that the penetration hypha is very slender, but even so it is inconceivable that the reaction to this thrust can be taken up by the germ-tube unless the latter is fixed to the epidermis. In this connection it might be pointed out that some of the figures which have been put forward as illustrating mechanical penetration can have no such meaning. Germ-tubes are figured as pointing down into depressions of the epidermal wall, and these



depressions have been cited as furnishing proof of mechanical pressure by the fungus. Obviously they are artifacts due to distortion of the material in preparation. It is to be remembered that the thrust is exerted and reaction to the thrust is taken up within the minute area common to appressorium and epidermis, and, therefore, any visual demonstration of pressure exerted is hardly to be expected.

The importance of the outer layer of the plant's body, whether cuticularized epidermis or cork, in preventing the entrance of parasitic fungi is well recognized. Thus there are many organisms ("wound parasites" such as *Stereum purpureum*, *Nectria galligena*, etc.) which strongly parasitize certain plants, if introduced into the tissues. The undamaged plant is, however, immune to attack. Again the strengthening of the cuticularized epidermis which takes place with advance to maturity often leads to increased resistance. For example, the attack of apple leaves by the scab or the rust fungus is much more vigorous with young than with older leaves, though in this case it is not quite certain that a progressive increase of internal resistance has not also taken place. Similarly the greater susceptibility of etiolated and "forced" plants as compared with ones grown normally is due, in part at least, to the poorer development of their protective layers. Of specific references to the subject of epidermal resistance, the following will serve. Melander and Craigie (96) have shown that the resistance to penetration, as measured by a mechanical device, of the epidermis of certain *Berberis* spp. is correlated with resistance of the plants to the basidiospores of *Puccinia graminis*. Curtis (31) and Valteau (132) have found that the resistance of plum varieties to the brown-rot fungus is correlated with thickness of the skin. According to Graf-Marín (57), the increased resistance of older barley plants to mildew attack is due to increased thickness of the cuticle. If the latter is shaved off the plants are readily infected.

The behavior of the stomata in the matter of the times of their opening and closing is claimed by some observers to determine the resistance or susceptibility of plants to certain parasites. As night, with its deposits of dew, is the period when fungal spores are liable to germinate, it is understandable that plants with stomata which remain closed until well after sunrise may escape infection, possibly because the closed stoma prevents entrance or because no

chemotropic influence is forthcoming. The evidence, however, is somewhat conflicting. Such "functional resistance" has been described for certain wheat varieties against invasion by *Puccinia graminis tritici* (Hart, 62) and earlier by Pool and McKay (111) for mature beet leaves against *Cercospora beticola*. According to Hull (71), susceptibility of maize varieties to *Puccinia sorghi* runs parallel with the number of stomata per unit area of the upper surface and a somewhat similar, though incomplete, parallelism has been indicated for the response of rice varieties to *Piricularia oryzae* (Nagai and Imamura, 100). On the other hand, little or no correlation between stomatal features and resistance is reported by Ward (138) for species of *Bromus* and *Puccinia bromina*, by Goulden and his co-workers (56) for wheat and *P. graminis*, by Radulescu (112) for wheat and *P. glumarum* and by Lepik (89) for the vine and *Plasmopara viticola*. The issue has been further complicated by the recent statement of Caldwell and Stone (22) that the germ-tube of *Puccinia triticina*, on coming over a stoma and producing the so-called "appressorial vesicle," causes the stoma to shut. The germ-tube is, nevertheless, able to enter the closed stoma. The whole subject of functional resistance is thus still rather obscure.

*After Penetration.* The study of the interaction of host and parasite, once the latter has entered, is of great fundamental interest from the complexity and variety of the effects shown. At this point it will be convenient to treat the facultative and the obligate types of parasite separately, for though the distinction between them is not hard-and-fast and though they possess certain features in common, nevertheless there are important dissimilarities which stand in the way of a uniform treatment. This applies particularly to the experimental methods which are available in the study of the respective types, as will appear shortly.

#### THE FACULTATIVE PARASITE

A sketch of the mechanism of this type of parasite has already been given and it now remains to amplify that account and to discuss the mechanisms employed by the plant in resisting fungal attack.

Briefly stated, the facultative type of parasite, illustrated by such a typical example as *Botrytis cinerea*, invades the tissues of the host by excreting a destructive principle which kills the cells and more

or less dissolves the cell walls in advance of the position of the hyphae. The fungus is thus living all along as a saprophyte, and in accordance with this feature one finds that such fungi can be cultivated, with greater or less ease, on artificial media. One is, therefore, in a position to study their metabolism under strictly controlled conditions, at least up to the limits set by chemical technique. Again, since the fungus in process of attack is living on dead parts of the plant, the physiology of its parasitism is the sum total of the physiology of its nutrition and of the mechanism whereby the living tissue is broken down by the fungal excretions. The problem thus presents no insuperable difficulties, and it is not surprising, therefore, that this type of parasitism has been the most fully explored.

The offensive mechanism possessed by the parasite consists of the products which it is able to excrete and it would be unsafe to disregard any one of these in this connection. Such products include enzymes, especially those which attack cell wall constituents, organic acids, alcohols, and many more. Chief attention has been paid to enzymes and organic acids, as has already been indicated, and varying importance has been attached by one author or another to each. Thus the writer claims that the enzymic system, in particular the pectinase, of *Botrytis cinerea* produces on the host tissue all the effects of which the fungus itself is capable. Nevertheless, it would be rash to claim that what is true of one fungus is true of others, so that there is no necessary opposition between the writer's work on *Botrytis cinerea* and that of Clayton (27) on *Bacterium tabacum*, and of Pierstorff (110) on *Bacillus amylovorus*, of Higgins (67) on *Sclerotium rolfii* and of Johann, Holbert and Dickson (74) on *Penicillium oxalicum*. Clayton and Pierstorff ascribe the toxic action to an unspecified thermostable substance, Higgins and Johann *et al.* to oxalic acid.

In work of this description it is important to remember that the metabolic products of an organism may be widely different on different media. Hence an experimental difficulty arises in making a sufficiently close approximation to the medium in which the parasitic fungus is actually growing, viz., the tissue of the host which has been killed by the fungus. Clayton's claim for a thermostable toxin will be more readily accepted when he has shown that it is excreted by the organism as grown on tobacco leaf tissue, and, as the toxin

is apparently a relatively simple substance, when he has proceeded some distance in its identification. The claims in favor of oxalic acid are, in the writer's opinion, open to serious criticism. A careful study of the two papers cited does not give any clear proof that a toxic concentration of oxalic acid was present in the region where the fungus was invading. That a large amount of oxalic acid is produced by such and such a fungus on a particular medium is not relevant evidence, since the formation of this acid is notoriously dependent upon the composition of the medium and other cultural conditions. Also the occurrence of crystals of calcium oxalate in the parasitized tissue, to which reference is made by some workers, does not in itself show that a toxic concentration of oxalic acid is present, but rather that the amount of free oxalic acid has been kept at the vanishing point until all the soluble calcium salt has been precipitated.

The general criticism offered in the foregoing paragraph applies also to the writer's work on the enzyme of *Botrytis cinerea*, but this work has at least the advantage that certain *à priori* considerations are in its favor. In any disease where there is definite rotting of tissue the existence of a cell-wall dissolving enzyme must be postulated, since there is no plausible alternative. Extracts of very high activity have been obtained by special methods, but there is no need to postulate such a high concentration of enzyme within the tissue, since the action of the fungus is much slower than that of the extracts. The enzyme being a catalyst, a weak concentration will produce the same effect as a stronger if given sufficient time, whereas with oxalic acid the toxic effect is determined largely by the concentration.

In addition to the quantitative requirements just indicated, qualitative effects must be borne in mind. The solution of the active principle should reproduce the action of the fungus in detail, or if not, the divergence should be explainable. Judged by this criterion, some of the work on oxalic acid fails. Thus Smith (124) claimed that the toxic and macerating effects of *Botrytis cinerea* are due to oxalic acid. Apart from a complete lack of evidence that oxalic acid was produced by the fungus, there was the striking point that the acid bleached the killed tissue whereas the fungus and the fungal extracts turned it into a reddish brown. Smith's explanation of this difference is untenable. The reddish-brown

coloration is the natural post-mortem change in lettuce tissue, whenever the conditions of killing have been such as not to destroy the oxidase coloring system of the plant. The fact that oxalic acid interferes with this reaction, whereas the fungus does not, is sound evidence that oxalic acid is not the active constituent of the fungal apparatus.

The writer does not favor the theory that oxalic or any other organic acid is of prime importance in connection with fungal invasion, though it may be that in some cases an acid reaction markedly favors the progress of attack, as for example with *Penicillium* spp. on orange fruit (Green, 59). It is noteworthy that the fungus *Aspergillus niger*, forms of which are able to produce large quantities of oxalic acid, is not remarkable for its parasitic tendencies. Furthermore, many plant diseases are characterized by the development of an alkaline reaction, so that there can be no question with these of the participation of an acid. Finally, any theory based on the toxicity of organic acids would seem to be quite inadequate when one remembers the inverse relationships so abundantly illustrated in pathology. Fungus  $\alpha$  attacks host  $A$  but not host  $B$ ; fungus  $\beta$  attacks  $B$  but not  $A$ . How can this be explained on the acid theory? The conditions governing acid formation in culture are fairly well known, and one of these is the presence of a large amount of readily oxidizable carbohydrate. Judged by the behavior of fungus  $\alpha$ , the tissue of  $A$  is more favorable to acid production than is the tissue of  $B$ ; while the converse is true if one considers fungus  $\beta$ . The acid theory is too simple, and on that account unpromising.

The enzymic theory is not free from considerable difficulties. Thus Harter and Weimer (63) found no correlation between the parasitic vigor of some species of *Rhizopus* and their capacity to excrete pectinase on certain standard media, a conclusion which was confirmed by Paul (107) for a number of strains of *Fusarium fructigenum*. The enzyme is also known to be secreted by a number of saprophytic fungi and bacteria. These difficulties have been largely cleared away by recent work in the writer's laboratory, where a systematic study of the factors governing the secretion and functioning of the enzyme is in progress. A review of this work has been given elsewhere (21) so that a summary will suffice here. It has been found that the composition of the cultural medium affects the amount of enzyme secreted, so much so that in media with

a high preponderance of carbohydrate over the nitrogenous constituent very little enzyme is produced though there is considerable mycelial development. Conversely, in media with low carbohydrate and high nitrogen content growth may be poor but enzyme secretion is active. Furthermore, the detailed properties of the enzyme, such as sensitiveness to H-ion concentration and to various retarding chemical agents, vary from one fungus to another and even for the same fungus according to circumstances (25, 97). There is clear evidence that the properties of the enzyme are determined to some extent by the presence of some other substances. In the light of these results it is clear that no correlation between the amount of enzyme formed on any selected medium and the parasitic vigor of the fungus need be expected. A further point of considerable significance is that a strong solution of pectinase enzyme, if added in limited quantity, may be rendered inert by some action of living tissue. In a comparative study of *Bacillus carotovorus* and *Pythium de Baryanum* which attack potato tissue and of a strain of *Botrytis cinerea* which does not, results of the type summarized in Table I are obtained. The tissue of potato tubers has normally a certain avidity for water; such tissue is referred to in the Table as "subturgid." Previous to the experiment the enzymic preparations are tested by a standard method and their activities adjusted to the same value. Equal small quantities are then placed upon cylinders of potato tissue.

TABLE I

|  | <i>Tissue subturgid</i> | <i>Tissue turgid</i> |
|--|-------------------------|----------------------|
| <i>Botrytis</i> spores .....           | No attack               | Attack               |
| <i>B. carotovorus</i> suspension ..... | Attack                  | "                    |
| <i>P. de Baryanum</i> mycelium .....   | "                       | "                    |
| <i>Botrytis</i> enzyme .....           | No attack               | "                    |
| <i>B. carot.</i> " .....               | Attack                  | "                    |
| <i>Pythium</i> " .....                 | "                       | "                    |

The same result is obtained even when the *Botrytis* enzyme is definitely more active, as tested by the standard method (on turgid tissue), than those of the other two organisms. The enzymes of *B. carotovorus* and *Pythium* are more active upon the turgid than on the subturgid tissue, as likewise the organisms, but the signifi-

cant point is that while a certain degree of lack of turgor slows down the activity of *B. carotovorus* and *Pythium* and of their enzymes, the same inhibits *Botrytis cinerea* and its enzyme. The striking parallelism between the behavior of organism and enzyme gives grounds for the belief that the mode of action of the organism will be much illuminated when the mode of action of the enzyme is better understood. The enzyme theory manifestly involves many complications, but one could hardly expect that a complicated set of phenomena would be explainable on any simple theory.

Leaving now the subject of chemical means of attack, one cannot ignore the possibility that fungi may be able to progress through a tissue by mechanical action alone. Cell walls might be pierced by mechanical means and the mere presence of a foreign body, such as a hypha, within the protoplast might conceivably lead to death of the latter. The extent to which mechanical or chemical factors come into play in the progress of invasion probably varies very much in different instances. Where there is pronounced rotting of the tissue, chemical action is no doubt predominant, and conversely, where microscopic study shows that the fungus has traversed cell walls by means of fine penetration hyphae, one is justified in speaking of mechanical penetration. There are some references in the literature to the correlation of resistance with mechanical hardness of tissue or with average thickness of cell walls—as in the case of plum varieties to *Sclerotinia cinerea* (144) and of potato varieties to *Pythium de Baryanum* (65). On the other hand, bacterial parasites, for which it is difficult to postulate a capacity to penetrate cell walls mechanically, do freely permeate the tissue of the invaded plant, thus indicating that the process of invasion within the tissue can be effected by chemical means alone. The same explanation would apply also to the penetration of cells of potato tissue by the plasmodium of *Spongospora subterranea* as described by Kunkel (84).

The aggressive mechanism of the parasite having now been discussed, it remains to consider the nature of the resistance offered by the plant. This falls under the usual two headings, mechanical and chemical.

Layers of cork formed some little distance in advance of invading hyphae are the most familiar examples of the mechanical type of resistance. These are constant features in certain diseases,

e.g., in apple-scab and in the corky-scab disease of potato, at least in the milder form of the latter. In both cases the cork barriers are considered to function in preventing the invasion of the deeper tissue layers by the parasite. In a study of the resistance of different varieties of pear to *Bacillus amylovorus*, Shaw (121) has found that the more resistant varieties are characterized by a more rapid formation of cork round the bacterial lesions and Fahmy (41) states that the recovery of cotton plants from attack by *Rhizoctonia solani*, which is brought about by raising the temperature, is accompanied by the walling off by a corky layer of the cavity formed by the fungus. Nevertheless, there has always been some doubt as to whether cork barriers really function at all or merely mark the limit of spread of the parasite which has already been stopped by some chemical factor. Thus, Cunningham (30) reports that the margins of lesions caused by certain leaf-spotting fungi may or may not show corky barriers, though the advance of the parasite is equally arrested in both cases, and Thomas (128) notes that cork barriers may actually be penetrated by the hyphae of *Armillaria mellea*. The same considerations apply to the gum barriers which are associated with the resistance of certain plum varieties to silver-leaf disease (13) and of lettuce to *Botrytis* disease (1).

The formation of cork or of gum is a wound reaction which is characteristic of certain plants and which may be modified by the conditions prevailing, e.g., by aeration, by humidity, by the vigor of growth of the plant, etc. It is not clear whether fungal secretions influence the formation of cork or gum; whether, for example, a rampant parasite suppresses the natural tendency of the plant to lay down such barriers, or conversely, whether a fungus which has been arrested in its progress has contributed to that end by intensifying the natural response of the host. That the excretions of some fungi intensify the formation of wound gum has been suggested in Willison's (145) study of certain peach diseases.

Cells with lignified or otherwise altered walls are, in general, resistant to invasion, and in some cases the presence of such cells limits the area of spread of the parasite. Perhaps the most familiar examples of this structural type of resistance are found among the obligate parasites, e.g., as seen in the line-like sori of some rusts (*Puccinia glumarum*) or smuts (*Ustilago longissima*) on



grasses, where lateral spread of the fungus is prevented by the parallel strands of fibro-vascular bundles. Within the group of facultative parasites, the effect is well shown in the lesions produced on cotton leaves by *Bact. malvacearum* whence is derived the common name "Angular leaf-spot" disease of cotton. The endodermis, with its walls thickened on the radial surface, is likewise in some cases a layer resistant to invasion, as has been shown by Pearson (108) for varieties of corn resistant to *Gibberella saubinetii*.

The influence of chemical factors in determining internal resistance has been much investigated. An obvious line of research is to attempt to demonstrate, in the sap of resistant plants, the presence of substances inhibitory or toxic to fungi. Thus the occurrence of tannin (which is somewhat toxic to fungi) in the outer layers of plants has been considered (29) to predispose to resistance. Schmidt (120) finds that species of *Solanum* resistant to *Cladosporium fulvum* contain in their sap a principle antagonistic to the fungus. There was some indication that the active ingredient was the alkaloid solanin, but Schmidt found it necessary to postulate a hypothetical substance "prohibitin" of unknown composition. According to Reynolds (113), there is a higher percentage of a glucoside, which gives hydrocyanic acid on hydrolysis, present in flax strains which are resistant to *Fusarium lini*. Spores of *Fusicladium dendriticum* germinate better in the sap of susceptible than of resistant varieties of apple (146, 79) but curiously the amount of germination is better still in pure water. Thus one must conclude that susceptible varieties are attacked in spite of some antagonistic principle in their sap. This principle (79) is most abundant in trees deficient in nitrogen, which are known to be the most resistant, but it occurs in quantity also in young leaves and fruits, which are quite susceptible. The relation between resistance and composition of sap is, therefore, not very clear in this case.

Perhaps the most striking evidence of the association of resistance with the presence of a toxic compound is that furnished by Link and Walker (91) in their studies of the "smudge" disease of onions caused by *Colletotrichum circinans*. It is well known that raw onion juice contains a volatile principle which is distinctly toxic to fungi, but less so to those which parasitize onion. Over

and above that, these workers have shown that the sap of colored resistant varieties is more toxic to *Colletotrichum* than is that of white susceptible varieties. This greater activity has been proved to arise from the presence of two aromatic compounds, protocatechuic acid and catechol.

Acidity of the cell sap is claimed to determine the resistance of some plants. Thus Horne (70) associates the diminishing resistance of apple fruits which takes place during the process of ripening with progressive diminution of acidity. Similarly, Dickson, Link and Dickson (35) find that the embryonic tissues of corn varieties which are resistant to *Gibberella saubinetii* have a high acidity due to the presence of sugar acids.

An interesting type of chemical resistance is that in which the factor responsible is the low concentration of a sap constituent. Horne (69), for instance, has shown that there is a high correlation between low soluble nitrogen content of apple fruits and resistance to a number of parasites and this has been confirmed by Vasudeva (136) who finds that the invasion of apple fruits by such parasites as *Botrytis cinerea* and *Monilia fructigena* is much intensified by the supply of a trace of soluble nitrogen in the inoculum; and, furthermore, that a similar addition renders *Botrytis allii*, a non-parasite of apple, capable of attack. Apple fruit is very deficient in soluble nitrogen and Vasudeva explains the results just described on the ground that low nitrogen depresses the secretion of pectinase enzyme by the fungi. Conversely, a high nitrogenous content predisposes to attack, as has been shown by Böning (10) for *Bacterium tabacum* on tobacco leaves and by Fehmi (43) for a number of rotting organisms on potato tubers.

An interesting elaboration of the tannin hypothesis of Cook and Taubenhaus (see above) has been propounded by Dufrénoy (37) in a series of papers. This author has examined cytologically the effects of a number of parasites (obligate as well as facultative) upon plant tissues and claims that resistance arises from the accumulation, in cells bordering the lesion, of tannins or other phenolic derivatives. The accumulation of an excessive amount of these toxic substances is stated to cause the death of the plant cells and also of the parasite. The evidence is based on staining reactions of vacuolar contents, and while the appearance described seems to be widespread and on that account must be allowed to be

of importance, it is not easy to discover any solid basis for the conclusions drawn. Certain dark staining masses are stated to be phenolic compounds and the concentration of these is said to be sufficient to kill host-cells and parasite. Clear evidence bearing on each of these points is required. In so far as Dufrénoy's work explains the arrest of fungal lesions, two criticisms of an *à priori* nature may be offered. In the first place, if the mechanism of arrest is as stated, it is difficult to understand why the lesion ever progresses at all beyond microscopical dimensions, and in the second place, it is open to question whether, at the time a fungal lesion has reached its utmost limits, the hyphae of the parasite are in fact dead. In the light of what is known about the growth of fungi in culture, it is more plausible to suggest that the arrest of a fungal lesion, if it is not due to an environmental change which is unfavorable to attack, is to be explained by the stalling effect of the products of fungal metabolism.

While examples of antagonistic chemical factors could no doubt be multiplied, many investigators have failed to detect any correlation between resistance and the acidity or any other property of the cell contents, and one may venture to forecast that this will prove to be the general rule. At the best, one can only visualize inhibitory or toxic factors as restricting the number of parasites possible. Thus it is understandable that only fungi (e.g., *Monilia fructigena*) which prefer or at least tolerate a high degree of acidity could parasitize very acid tissues (such as young apple fruit). At the same time there is a vast number of plants with sap acidity which is well within the range of a large number of parasitic fungi—which nevertheless may be unable to parasitize these plants; and the same applies to other toxic or inhibitory constituents of the cell sap. What mechanism, therefore, can be suggested to explain a type of chemical resistance which is independent of substances antagonistic to fungal growth? Two lines of research may be quoted in this connection.

The behavior of potato tissue to *Botrytis cinerea*, to which reference has already been made, illustrates a type of resistance which is probably of very wide occurrence. Here there is nothing in the composition of the tissue which is harmful to the fungus. The spores germinate strongly in extracts of the tissue by whatever method these are prepared. These extracts are entirely suitable

for the development by the fungus of its offensive mechanism. Nevertheless, there is no attack. As was pointed out above, the line dividing attack from non-attack is very narrow, since a slight increase in the water content changes the tissue from resistant to susceptible. The explanation is probably to be found in the mechanism of enzyme action, as was indicated above.

Though it is not proposed in this article to deal at length with the effect of environment on disease, a few words may appropriately be said at this point on the effect of water relationships. That a high degree of humidity is generally a factor which favors plant disease has been long known, but the usual idea is that humidity is important chiefly in the phase before penetration. The discussion of the foregoing paragraph indicates how reduced water content may contribute to resistance by its effect upon the enzymic mechanism. A number of statements in the literature clearly indicates the close relationship between high water content and susceptibility; e.g., of pear to *Bac. amylovorus* (121), of tobacco leaves to *Bact. angulatum* and *Bact. tabacum* (28) and of potato leaves to *Phytophthora infestans* (101). It is probably for the same reason that certain workers, e.g., Young (149), have found that under artificial conditions of inoculation a number of fungi attack hosts upon which they do not occur naturally, so that one may speak of "new diseases" produced in the laboratory.

The second line of research, which considers a type of chemical resistance which is independent of plant toxins, is that of Dickson and his collaborators (34, 36) on the reaction of wheat and corn varieties to *Gibberella saubinetii*. The same strain of this organism attacks varieties of both wheat and corn, but with the difference that wheat is attacked at high but not at low temperatures, whereas with corn the converse relationship is shown. Dickson's work indicates a striking correlation between resistance and a type of metabolism which is in the main determined by the prevailing temperature. At low soil temperatures, the starch of the wheat endosperm is hydrolyzed much more rapidly than is the protein, with the result that the seedling is rich in sugar but poor in nitrogen. The cell walls, therefore, thicken rapidly by the deposit of cellulosic material upon the original pectic framework, and on that account become much less susceptible to fungal attack. On the other hand, at high temperatures both the starch and the protein

are rapidly hydrolyzed, the seedling is definitely richer in soluble nitrogen, growth is much more rapid and the cell walls remain much longer in the primary pectic condition. Hence the greater susceptibility to fungal attack. The behavior of corn seedlings in relation to temperature is the converse of that described for wheat. At high temperatures (which are favorable to the corn plant) the cell walls of the seedling are of a resistant and somewhat suberized type, whereas the unmodified pectic type of cell wall is produced at low temperatures.

More recently Gäumann (54) has confirmed that the cell walls of wheat germinated at a high temperature are more easily hydrolyzed by *Fusarium herbarum* than those of plants grown at a low temperature, and that the curve of digestibility of the cell walls runs parallel to the curve of their pentosan content. These investigations all point to the importance of cell wall composition in determining resistance and reinforce the writer's view of the importance of the pectinase enzyme in parasitism.

The discussion of the facultative type of parasite has so far referred to the kind of disease in which the effects upon the host are more or less confined to the neighborhood of the parasite. There is, however, a well known class of diseases in which the main symptoms tend to be shown by the whole plant and at parts more or less remote from the location of the parasite. These are the important wilt diseases, which now call for some special consideration.

The essential feature of a wilt disease is that the parasite enters the root system and after traversing the cortex, in which it may produce relatively little disturbance, it reaches a striking development in the vascular system. The functioning of the latter is interfered with, and as a result the shoot wilts and the whole plant dies. All manner of views have been expressed as to the cause of wilting and killing. The earliest investigators (123) suggested that the cause was simply mechanical blocking of the water-conducting system by the fungal mycelium. Further study of the many naturally occurring wilts showed that this explanation was inadequate, for in many cases there is relatively little mycelial development within the vessels. While theories of mechanical blockage (by tyloses, gum formation) have not been altogether abandoned, the general tendency has been to search for a toxic sub-

stance which is excreted by the fungus into the water stream of the plant. The method of experiment has been somewhat as follows.

Cultures of the parasite are set up on a liquid medium\* and after a certain time the fungal mat is filtered off and the filtrate examined for toxic substances by placing in it whole plants or cut shoots of the host. Parallel controls are set up with water or with various strengths of the original culture medium. Extracts of the mycelial mats have been similarly tested. The presence of a toxin is shown by the wilting of the test plants.

A selected list of the conclusions which have been reached by various workers is set out in Table II, from which it will be seen

TABLE II

| Host: Parasite   | Cause of Wilting  | Author                        |
|--|---|-------------------------------|
| Melon, <i>Fusarium nivium</i> .....                    | Tyloses   | Sleeth (122)                  |
| Tomato, <i>Bact. solanacearum</i> .                    | Gum formation   | Van der Meer (133)            |
| Cotton, <i>F. vasinfectum</i> .....                    | Rotting of roots  | Fahmy (40)                    |
| " " " .....  | Physiological;<br>Aluminum salts<br>from soil                     | Dastur (32)                   |
| " " " .....  | Volatile alkaline<br>substance + nitrite                          |                               |
| " " " .....  | Thermolabile<br>substance   | Rosen (117)                   |
| " " " .....  | Thermostable<br>substance which<br>is not nitrite                 | Neal (102)                    |
| " " " .....  | Amines  | Fikry (44)                    |
| Tomato, <i>F. lycopersici</i> .....                    | "   | Schaffnit and<br>Lüdtke (118) |
| " " " .....  | Enzyme + volatile<br>substance + ther-<br>mostable sub-<br>stance | White (141)                   |
| " " " .....  | ? Ammonia   |                               |
| " <i>Verticillium albo-atrum</i>                       | Nitrite   | Luz (93)                      |
| Flax, <i>F. lini</i> .....                             | CO <sub>2</sub> gas bubbles                                       | Van der Veen (134)            |
| " " " .....  | Thermostable<br>substance   | Tochinai (131)                |
| Banana, <i>F. cubense</i> .....                        | Aldehyde  | Grossmann (60)                |
| Sugar cane, <i>Helminthosporium<br/>sacchari</i> ..... | Thermostable<br>substance;<br>? nitrite                           | Lathrop (85)                  |
| Plum, <i>Stereum purpureum</i> ...                     | Enzyme + thermo-<br>stable substance                              | Lee (88)                      |
|  |   | Brooks &<br>Brenchley (12)    |

\* Richards' solution (cane sugar 50 g; KNO<sub>3</sub>, 10 g.; KH<sub>2</sub>PO<sub>4</sub>, 5 g.; MgSO<sub>4</sub>, 2.5 g.; FeCl<sub>3</sub>, trace; water, 1 litre) or some modification of it (e.g., glucose in place of cane sugar) has been much used in these studies.

how very diversified are the views expressed. Included in the table are two diseases (the last two) which are not classed as wilts, but as they both show the same "action at a distance" and as the mechanism of this action has been studied along the lines indicated, their inclusion in the table is quite appropriate.

Some of the work has been carried out with great care, e.g., effects which might arise from changes of H-ion concentration in the culture medium have been eliminated, and the test solutions have been freed from fine suspensions (which might block the vessels) by filtration through porcelain. It is difficult, therefore, to avoid the conclusion that destructive substances are present in the fungal filtrates. Very few workers, however, have studied the effects on the plants in detail, and it is not clear whether the action is on the vascular elements or on the living cells of the leaf. It would be interesting in all cases to know whether the wilted shoots were in fact poisoned or were merely wilted on account of some interference with the ascent of water.

Some workers, e.g., Haymaker (66), claim that the action of the toxin is specific and closely parallels that of the fungus on the host. Thus the filtrates of virulent strains of the fungus are the most active, and susceptible hosts are more sensitive to the toxin than resistant ones. This view, however, is exceptional and the general consensus is that there is no specificity. Not merely do resistant varieties succumb to the toxin as readily as do susceptible varieties, but so also do plants which are in no way connected botanically with the particular host species. This lack of specificity is not in itself a real objection to the toxin theory as it is quite plausible that wilt resistance is based on some character of the host other than resistance to the toxin. Perhaps a more serious objection to the toxin theory is contained in the results of Barnum (5) who finds that the same wilting effects are produced by filtrates of *Penicillium expansum*, which is not a wilt-producing fungus.

The production of toxins in culture is not an invariable feature of a wilt-producing fungus, as was recognized by Rosen (*l. c.*), one of the earlier protagonists of the toxin theory. He found that the cotton-wilt fungus gave toxic filtrates when grown in Richards's solution but not in Uschinsky's solution or in a number of natural decoctions. In this connection he writes: "This

opens up the whole question as to the significance to be attached to findings of toxic properties with any medium which does not closely approach the chemical and physical make-up of the natural host." In the light of this statement it is curious that he bases his claims on filtrates obtained from Richards's solution, which is not obviously a close approximation to the sap of the root cells or of the vascular system. This question of toxins and the relevancy of the findings of cultural experiments has already been considered for a simpler type of host-parasite relationship. In the case of wilt diseases there is the further complication that it is not clear which living cells in particular are being poisoned by the toxins, whether cells of the root cortex, or living cells associated with the vascular system in the stem or the mesophyll cells of the leaf. It should be possible to provide evidence on these points, but there would still remain the serious difficulty of trying to understand the breakdown of a process, viz., the ascent of sap, when plant physiologists have not yet produced a completely satisfactory theory of its mechanism in the normal plant.

The toxin theory of wilt diseases loses in cogency from the multiplicity of toxic agents suggested (Table II) and from the claims that fungi which do not cause wilting diseases, nevertheless, produce toxins. It is not improbable that the mechanism envisaged, viz., the excretion by the fungus of a toxin or toxins into the vascular stream, presupposes an over-simplification of the problem. Wilt diseases are not at all uniform in kind. In some there is apparently little damage to the root cortex, but in others there is very definite root rotting, and there is in fact no hard-and-fast line between a wilt and a root-rot or foot-rot disease. The same organism may cause a cortical rotting of a young plant and a wilt of an older plant. The "action at a distance" feature is not always shown as some wilt-producing fungi are not confined to the lower parts of the plant. The wilting and killing of the host in some cases follow soon after inoculation, in others after a very long interval. Thus in the carnation wilt caused by *Verticillium cinerescens* (142) the incubation period may extend to many months and the whole plant may be permeated by the fungus before any external symptoms are shown. With such a variety of host-parasite relationships grouped together under the name of wilt diseases, it would not be at all surprising if the causes of wilt-



ing and death were somewhat different in different instances. Linford (90) has clearly shown the necessity of a much broader physiological treatment of the problem than would arise from the assumption that the action of the fungus is primarily on the water-conducting system.

One leaves the toxin theory of wilt diseases with the certainty, therefore, that no clear indication has been given of the nature of the toxin and with a suspicion that the complexity and heterogeneity of wilt diseases have not been fully appreciated.

Whatever the nature of the offensive mechanism of the parasite, it is generally agreed that the resistance of the host lies in the cortical cells of the root. This has been demonstrated, for example, by Tisdale (130) for flax wilt and by Jones (80) for the bacterial wilt of alfalfa.

#### THE OBLIGATE PARASITE

The contrast between a facultative parasite like *Botrytis cinerea* and an obligate parasite such as *Puccinia graminis* is very pronounced, and is as shown in the scheme below:

| <i>Botrytis cinerea</i>  | <i>Puccinia graminis</i>   |
|--|--|
| Host range wide .....  | Host range narrow.   |
| Hyphae inter- and intracellular .....  | Hyphae intercellular with intracellular haustoria.                   |
| Tissues of host killed in advance of fungal growth, <i>i.e.</i> no symbiosis shown ..... | Host tissue not killed until a late phase, symbiosis shown at first. |
| Parasite readily cultivable on artificial media ..                                       | Parasite non-cultivable on artificial media.                         |

A further general feature of the obligate parasite, though this is not shown by *Puccinia graminis* on its grass host, is the tendency to gall-formation, which is not nearly so marked with fungi of the type of *Botrytis cinerea*.

None of the differences listed above, however, is hard-and-fast and many intermediate conditions are known. As regards host range, some rust fungi are much less specialized (e.g., *P. malvace-*

*arum*, which ranges over several genera of the Malvaceae), and the parasitic range of the *morphological species* may be very wide, as in *Erysiphe polygoni* which attacks *Polygonum*, clover, pea, swede, etc. It is true that the species in the latter case consists of a number of biological races, each with a comparatively circumscribed host-range but the same is to a large extent true of typical members of the facultative group. Even *Botrytis cinerea* and *Rhizoctonia solani*, which are stock examples of the "plurivorous" type of parasite, consist of a large number of distinct races (or even subspecies) each of which has a much narrower range than that of the "group species." Then again forms like *Ophiobolus graminis*, *Venturia inaequalis*, *Cladosporium fulvum* and many others, which are classed as facultative parasites\* are as narrowly restricted in their parasitism as many members of the obligate group.

The relation of the mycelium of the parasite to the host cell gives one of the best criteria for distinguishing the two types. Where the thallus of the fungus is entirely intracellular (as in *Synchytrium* or *Plasmodiophora*) or where haustoria are the only intracellular structures (as in some downy mildews and smut fungi, in all powdery mildews and rust fungi) one may be certain that the parasite is of the obligate type.

Early and rapid killing of the host tissue is not always so pronounced as with *Botrytis cinerea* and in some cases killing is slow and not at all distinct at first. There are thus intermediate types which lead up to the condition shown by *Puccinia graminis* when growing in symbiosis with its appropriate host. Conversely, the "hypersensitiveness" shown by a host plant to certain strains of a parasite has been regarded as an effect peculiar to obligate parasitism, but it is claimed that something very similar occurs with *Colletotrichum lindemuthianum* or certain varieties of *Phaseolus* (86).

Non-cultivability of the parasite is associated with the haustorial habit. No great emphasis need be attached to this negative character, as it merely means that no one as yet has discovered the secret of a highly specialized type of metabolism. At the moment,

\* These are more appropriately described as "facultative saprophytes," in the sense that they have regularly parasitic phases but are able to live saprophytically in the intervals.

however, one can safely say that no fungus which possesses clearly defined haustoria has been cultivated on an artificial medium, that is, to any extent beyond mere spore germination. But here again there are intermediate states. Between the obligate parasite and the kind of fungus which is cultivable on any medium within reason, many intermediates may be noted. Thus *Exoascus*, which attacks its hosts after the manner of an obligate parasite, can be maintained in culture but almost wholly in a yeast-like form which is very different from its parasitic mycelium. Then among facultative parasites there are wide differences in the ease with which they can be cultivated. Some require special media, and if one is aiming at a complete life cycle in culture, with the characteristic fruiting structures such as perithecia, etc., the number of difficult subjects is very considerable.

Finally, the feature of gall formation is not confined to the obligate parasites and is not invariable in their case. Thus a distinct gall is formed round the lesion caused by *Nectria galligena*. *Fusarium moniliforme*, a fungus with pronounced killing tendencies, causes a peculiar "overgrowth" of the invaded plants as a preliminary symptom; and most striking of all, the crown-gall organism which is a readily cultivated parasite of wide host-range produces on its hosts large and characteristic galls.

The fact that the obligate parasite cannot be cultivated apart from its living host forces upon the investigator an indirect method of studying its physiological relationships. Its metabolism being quite unknown, one has to fall back upon microscopic examination the results of which may in part be interpretable in terms of physiology, and upon a study of the host's metabolism in its relation to the progress of the disease. The cytological relations of host and parasite have already been dealt with in this REVIEW (114). It now remains to consider the problem from the second point of view.

Most of the work in this connection has been carried out with rust fungi and more particularly with forms of *Puccinia* on cereal hosts. The method adopted is to study the effect of various environmental factors upon the grade of infection. Maximum susceptibility is correlated with best development, both as regards number and size of the spore masses, and with a minimum period from inoculation to sporulation. When the host is resistant, sporulation,

if it occurs at all, is scanty and the spore masses are small; there is also in many cases a development of minute dead spots over the surface of the host, this being the outward expression of "hypersensitiveness." A series of infection types, ranging from highly susceptible to highly resistant or immune, has been distinguished by various workers on the basis indicated.

The general effect of *light* upon the host is to increase susceptibility, though the existence of an optimum period of illumination has been indicated (8). Forward (45) states that for *Puccinia graminis tritici* the uredo-pustules are fewer in number and slower in development when the host is kept in darkness, and that in poor light there is a tendency towards hypersensitiveness in hosts that are normally quite congenial to the parasite. Similar results have been obtained by Gassner (48), Johnson (75) and Wilhelm (143) for various cereal rusts and by Waters (140) for bean rust. The association of susceptibility with good conditions for assimilation is further supported by Hanna's (61) statement that wheat varieties which are highly susceptible to *Puccinia graminis* are characterized by a relatively high content of the green and yellow chlorophyll pigments.

In this connection it is interesting to note the behavior of the nodule bacteria of lucerne and clover when the host plant is kept in darkness. Under such conditions the bacteria do not set up the normal symbiotic relationship but actively parasitize the root cells (129).

The influence of *temperature* upon the infection type has been studied for a number of rust fungi, but with no clear-cut result. In many cases, change of temperature produced no effect, and where there was a change in host reaction it was not always in the same direction for the different diseases studied.

Considerable attention has been paid to *mineral nutrition* as a factor influencing susceptibility, and the general result has been to confirm the earlier work of Spinks (125) on this subject. High nitrogenous manuring, according to Spinks, increases the susceptibility of wheat and barley to their particular rusts and mildews, whereas potash acts in a contrary manner, though high nitrogen does not completely counteract the effect of high nitrogen. Conversely, plants with very low nitrogen are resistant even with minimal potash. The subject has been recently investigated in

great detail by Gassner and coworkers (50, 51, 64), by Schaffnit and Volk (119), and by some others, with results which are in essential agreement with the above. The effect of phosphorus is not always clearly marked; in some cases it acts in the same manner as potash and in others not.

An interesting observation made by Spinks but which does not seem to have attracted much attention, is that the addition of lithium salts markedly increases rust resistance.

The effect of *carbon feeding* runs parallel, as might be expected, to that of light. By placing the host plants (oats and wheat) in an atmosphere devoid of carbon dioxide, Gassner found that the development of the corresponding rust uredospores was inhibited. Gassner and Straib (49) found that the optimum concentration of carbon dioxide for the development of a number of rusts ranged from .15% to .75%. Concentrations of that order had no effect on the germination or growth of uredospores, so that presumably the effect on the disease arose from the action of the carbon dioxide upon the host. Results which point in the same direction have been obtained in another way. Waters (140) tested the susceptibility to *Uromyces fabae* of bean leaves which were fed with various sugar solutions, and found that uredospores were formed freely when the leaves were floated in the dark on a 5% sucrose solution whereas none were formed when the leaves were floated on water. This result has been confirmed by Yarwood (148), working with the rust and mildew of clover. The latter also states that detached leaves are more susceptible if taken from the plant in the late afternoon, when they are fully charged with carbohydrates than when taken in the early morning. All these statements support the view that active carbon assimilation increases susceptibility to the obligate type of parasite.

J] *The age of the host plant* has an effect, within limits, upon disease response and there is general agreement that resistance increases as the plant grows older. Hence arises the "mature plant resistance," the effect of which is to make a particular variety of the host resistant to strains of *Puccinia graminis tritici* to which it is susceptible in the seedling stage (55, 56). Similar results have been obtained for the crown and stem rusts of oats (38) and for brown rust of wheat (78). According to Gassner (52), *Puccinia triticina* conforms with the rule as stated, whereas *P. graminis avenae* and *P. lolii* behave in the converse manner.

A striking statement in this connection is made by Newton and Brown (193) who injected uredospores of *P. graminis* into the young and still enclosed leaves of various cereals. Under these conditions not merely are the very young leaves of resistant varieties highly susceptible, but the young leaves even of oats and rye are attacked by forms of *P. graminis tritici*.

The composition of the host has been studied from many points of view, with results that are in many cases negative or indefinite. Acidity of the cell-sap does not seem to be related in any way to susceptibility (72, 126, 73). Newton, Lehmann and Clarke (104) find no correlation between resistance and various properties of the cell sap such as osmotic pressure, electrical conductivity, H-ion concentration, total solid content or amount of bound water. Even the sugar content does not appear to vary consistently with variations of resistance. Thus Schaffnit and Volk found that the susceptible type of plant which resulted from over-manuring with nitrogen had a high sugar content, and conversely for the resistant nitrogen-starved plant. Nevertheless, low potash gave a type of plant which had a low sugar content but which was highly susceptible. As from one variety of wheat to another, Johnson and Johnson (76) have found no relation between resistance and the sugar content of the sap. Ezekiel's (39) claim that better germination of uredospores takes place in the sap of susceptible than of resistant varieties has not been borne out by the later work of Anderson (2). From a study of the reaction of wheat to a number of rusts at different temperatures, Gassner and Franke (53) find that increased susceptibility runs parallel with the concentration of protein nitrogen, thus supporting the earlier statement of Honecker (68) that resistance of barley to *Erysiphe graminis* is correlated with low albumin and abundant starch.

The presence of toxins in resistant varieties has been advocated by a number of workers. Thus Kharbush (82) considers that resistance of wheat to *Puccinia glumarum* is due to histolysis of the chloroplasts, leading to the absorption of a toxic substance by the fungus. This worker supports Dufrénoy in stating that resistance is associated with the accumulation of phenolic compounds in the vacuoles of cells bordering the invaded region, and more recently Newton and Anderson (105) have expressed the same view. Similarly, Rochlin (116) claims that there is a direct connection

between the resistance of various crucifers to club-root disease and the amount of glucosides in the roots which on fermentation give rise to pungent mustard oils.

Though the effect is entirely beyond explanation, it is interesting to note that the resistance of a plant to a specialized parasite may in some cases be broken down by the presence of another parasite. Thus the presence of the endo-parasite *Tilletia tritici* increases the susceptibility of wheat to *Puccinia glumarum* (137). Similarly, the resistance of Warden wheat to *P. triticina* is lost in the presence of *Erysiphe graminis* (77). Among facultative parasites the same effect is known, as in the diminished resistance to blight of potato plants which are infected with virus disease.

Whatever be the chemical substances which determine host resistance or susceptibility, whether toxic substances which act against or food substances which act in favor of the parasite, it is quite clear from a number of researches that those substances do not freely move about the plant. This is true not only for obligate but also for facultative parasitism. Thus Leach (87) has shown that when a variety of *Phaseolus vulgaris* susceptible to *Colletotrichum lindemuthianum* is grafted on a resistant one, the two components react in the normal manner, thus showing that organic continuity has not led to the interchange of substances which affect resistance. The same result has been obtained by May (95) and by Roach (115) for the resistance of tomato to wilt and of potato to wart disease, respectively. But perhaps the most striking evidence in this connection is obtained from infection experiments with graft hybrids (83), where it is found that the layers of the composite plant react to specific parasites exactly as does the tissue of the species from which they originated. Even such an intimate association of tissues as occurs in graft-hybrids does not lead to any interchange of substances which materially affect disease response.

So far for the effect of the metabolism of the host upon the reaction of the obligate parasite. When we come to consider the mechanism of the parasite there is little to say and that little is derived from cytological observation. Penetration of cell walls is apparently by mechanical means only as there is no trace of a chemical action upon the walls and the neck of the haustorium is always a slender thread-like structure, such as one associates with mechanical penetration. It should be noted that the effect of potash in increasing

disease resistance may be partly due to its action in intensifying cell wall development. Lowig (92), for example, has found potassium silicate to be the most effective salt in increasing the resistance of cereals to mildew attack and correlates this behavior with the mechanical resistance of the epidermal wall. Of enzymes or toxins which act upon the protoplasts there is no direct evidence but one may surmise that hypersensitiveness has its basis in a toxin either excreted by the fungus or liberated from the host through an action of the fungus.

### 7/ CONCLUSION

This review of the physiology of the obligate parasite-host relationship, which has unfortunately resolved itself largely into a catalogue of negatives, may be concluded best perhaps by raising a number of general considerations which, though they may not throw any light upon the problem at the moment, may indicate where a solution is to be sought with greater prospect of success. The cereal rusts have strongly attracted many workers, partly no doubt because of their great economic importance and partly because of the great variety of material (races of host and parasite) available for experiment. Furthermore, it is generally assumed that the relationship of the obligate parasite to its host brings in factors which do not exist in the simpler type. To illustrate, it is usual to compare the behavior of such a fungus as *Botrytis* on a susceptible plant with that of *Puccinia* on a resistant plant, and to state the position in this form: that a physiological relationship which leads to susceptibility with the one type of parasite leads to resistance in the other. Susceptibility to an obligate parasite would, therefore, be something which finds no parallel in the story of the facultative parasite, and so the tendency has been to emphasize the contrast between the two types. In particular it has been customary to ascribe the parasitic specialization of the obligate parasite to its nutritional specialization, e.g., to fine preferences for isomeric forms of carbohydrates or proteins. Such specialization as the facultative type of parasite shows must obviously rest upon grosser differences.

Now it is hardly possible to maintain a difference in kind between the two types of parasitic relationship, in view of the intermediate types which are known to exist. Even the symbiotic phase, which is the most striking feature of obligate parasitism, finds clear parallels



among the facultative group, as in the case of mycorrhiza, *Bacterium radicola*, etc. Again, the contrast between the obligate parasite which attacks its host most vigorously when the latter is growing under optimal conditions, and the facultative parasite which is favored by a weak condition of its host is definitely overdrawn. Certain forms of powdery mildew, for example, are favored by conditions (e.g., shading or droughting) which are far from optimal for growth of the host. It is even stated that local injuries predispose certain plants to mildew attack. Conversely, the statement that a weakened condition of the host predisposes to attack by facultative parasites is based on observations with certain types of the latter and it is certainly not true for many members of the group. Lastly, apart from a certain difference in degree, the phenomena of host specialization are the same in the two groups. One may, therefore, suggest that the key to the specialization of the obligate parasite may be sought for hopefully in a study of the facultative group. The latter have the enormous advantage of being cultivable, so that something may be learned about their metabolism. In particular, it may be possible to explain why some members of the group rapidly kill the invaded host cells whereas others do not. Information on that subject would go far to explain the symbiotic phase of obligate parasitism. It would even appear that gall formation could best be studied with the simpler type of organism, with *Bacterium tumefaciens* in particular. The physiological investigations with the *Puccinia* type of parasite, as reviewed above, have cleared up certain points and at least have served to define the problem, but they must necessarily be cramped by the non-cultivable nature of the parasite, and as such they do not, in the writer's opinion, represent a natural and scientific approach to the problem of selective parasitism.

Though it has been contended above that the specialization of the obligate parasite is not necessarily based upon its specialized nutrition, the latter is, nevertheless, an extremely interesting problem in itself, though unfortunately there is as yet no clue to its solution. Various chemical substances and plant extracts do in a somewhat irregular manner affect the vigor of spore germination, but no kind of medium has been found which is of value in giving sustained mycelial development. In speculating as to what might be lacking from the ordinary media, one would hardly suggest min-

eral elements as being of consequence, partly because they are present in the ionic form however supplied, and partly because fungi are able to sustain a reasonable amount of growth on the merest trace of mineral elements. The carbon food is also not likely to be the determining factor, as it is difficult to believe that glucose is not a suitable form of carbohydrate for all fungi. There remains the nitrogen constituent and there is in this case some evidence of specific preferences. Some fungi are able to assimilate nitrate, others not. Some prefer ammonium salts to nitrate, while others do not grow well except with organic form of nitrogen. The so-called "peptone" organisms apparently require complex forms of nitrogen, but as was shown by Farries and Bell (42) for *Nematospora gossypii* this selectivity is based, not upon the necessity for a protein as such, but upon growth-promoting substances which are associated with impure proteins. These growth-promoting substances are widely distributed in living tissues, are stable, diffusible and in the main non-specific. They may, therefore, be ruled out of account in the present connection as they occur in all natural decoctions and would be able to diffuse into the fungal hyphae. It appears, however, from recent plant physiological work, that a certain type of growth-promoting substance is readily deactivated by oxidation so that it does not occur in plant extracts unless certain precautions are taken (127). The functioning of such a labile substance offers a possibility which should not be ignored. Putting aside that possibility, one reaches the position that the obligate parasite requires to be supplied with nitrogen in its fully elaborated proteid form, and this approaches the view which is put forward by Gassner on the basis of his studies on rusts. On this view the haustorium would appear to be a structure which is permeable to a nitrogenous substance of high molecular weight, whereas the ordinary mycelium is not.

The speculations of the foregoing paragraph are based on the assumption that the obligate parasite is in fact highly specialized in its nutritional requirements, but it might be well to look at the evidence on which the current view rests. Briefly, it is that the germ tubes of obligate parasites do not respond to any known nutrient solution, whereas the parasitizing mycelium obviously obtains suitable food from the host. It is concluded, therefore, that certain food substances are made available by the living cells but that these are

not present in any extracts preparable from the latter. There is, however, another interpretation of the difference, which is that possibly the plant extract is quite suitable for growth *in vitro* but the fungus is presented in the wrong form—viz., devoid of haustoria. It is not unreasonable to suggest that if one could induce the formation of haustoria under artificial conditions, much of the so-called nutritional specificity might disappear. To be more explicit, one might suggest that the ordinary mycelium of the obligate parasite, being external to the plant cells, functions under strictly aerobic conditions and that the haustorium is a structure which is developed under reduced conditions of aeration (and perhaps in relation to some surface tension effect) and which absorbs food only under conditions of low oxygen tension. The latter suggestion is not so fanciful when one remembers that some enzymes are known which function only under these conditions. In this connection it might be interesting to study more fully the physiology of those fungi, e.g., certain Phycomycetes, which show a clear differentiation between an aerial and a matrical type of hypha.

A review of host-parasite relationships is not complete without some reference to the subject of induced or acquired immunity in plants. The orthodox plant pathologist is sceptical of the existence of such an effect and points out that, the plant body being organized on a basis essentially different from that of an animal, one cannot expect to find any close parallelism between plant and animal responses, and in particular that the phenomenon of generalized induced immunity cannot have a place in plant pathology. This view is supported by a vast amount of observational data showing that invasion of one part of a plant by a fungus in no way increases the resistance of other parts to subsequent reinfection. In recent years, however, some workers on virus diseases have described effects which at least closely simulate the effects which are well established by animal pathologists, and from time to time similar claims are made for plants diseases of fungal causation. Most of the latter claims relate to a localized acquired immunity, the inducing agent being some secretion of the fungus which is presumed to stimulate the host cells to a higher grade of resistance. The relevant literature has recently been reviewed in a favorable light by Chester (24). The subject is one of considerable complexity, for not merely are many of the results capable of more than one interpretation but there is still much

disagreement as to the results themselves. Any critical review of this subject would, therefore, of necessity be lengthy and would perhaps serve no useful purpose in the absence of general agreement as to what are fully substantiated data. The reader who is interested in this line of study will, therefore, be referred for further information to Chester's paper.

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# THE BOTANICAL REVIEW

VOL. II

JUNE, 1936

No. 6

## THE ABSORPTION OF ELECTROLYTES IN LARGE PLANT CELLS

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### INTRODUCTION

As this subject is most advantageously studied in large multinucleate cells the present review will be largely confined to such investigations.

The protoplasm of these cells forms a thin layer (not over 10 microns thick) outside of which is a cellulose wall and inside which is a large central vacuole filled with sap. At the inner and outer surfaces of the protoplasm are non-aqueous layers between which is an aqueous phase.

The advantages of such cells are manifold. Injury is easily detected (especial attention has been devoted to avoiding it in our experiments). The sap can be obtained with little or no contamination in sufficient quantities for analysis, without recourse to microchemical methods. Substances can be introduced into the cell without permanent injury by means of a capillary piercing the wall. In *Halicystis* two such capillaries were inserted by Blinks and left permanently in the cell so that the internal vacuole could be irrigated; *e.g.*, its sap was replaced by that of *Valonia* (of very different composition) and the cell continued to live (3). Another great

advantage is that an electric current can be sent through the capillary so as to pass only once through the living protoplasm.

In studying these cells new problems and points of view have been encountered. When we learned what was going on inside the cell we had to get rid of many preconceptions. For example, we soon found that the higher concentration of potassium inside the cell is not due to the formation of insoluble compounds (99) or to the Donnan equilibrium (p. 294).

Some puzzling facts have turned up which, perhaps, must wait for their interpretation until physical chemistry has made further progress, especially in relation to non-aqueous solvents. Certain principles, however, have become increasingly clear and some of these will be briefly discussed.

The main themes are: the kinetics of penetration, accumulation, selective permeability, and the nature of the protoplasmic surface.

#### THE KINETICS OF PENETRATION

This appears to be conditioned by the fact that the protoplasm has at the surface a non-aqueous layer, immiscible with water, through which entering electrolytes must pass. This is shown by a variety of evidence (99, 967), including electrical measurements on these and other cells.<sup>1</sup> An illustration of the latter is seen in the work of Fricke and Curtis (37) which shows that the surface of the yeast cell is composed of a non-aqueous layer of little or no conductivity. Rapidly growing yeast may take up a large quantity of electrolyte which must pass through this layer in the form of undissociated molecules, or of undissociated neutral complexes which carry no current and which, consequently, from our present point of view, are like molecules. For convenience we may, therefore, in the present discussion call them molecules. This may be illustrated by considering the penetration of acids.

*Acids.* It follows from what has been said that the rate of movement of a weak acid  $HA$  across the non-aqueous surface layer of the protoplasm will be proportional to the concentration gradient of its molecules in that layer. At the start of penetration we may neglect the internal concentration and may consider merely the concentration of molecules in the outer surface of this layer. This

<sup>1</sup> For work on these cells see 3, 4, 5, 6; bibliography in 99. For work on cell suspensions see 34, 35, 36, 37, 38; 24, 25, 26, 27.

will be proportional to the product  $(H)(A)$  in the external solution (under the simplifying assumption that there is no dissociation in the non-aqueous layer and that concentrations are equal to activities in all cases).

This is confirmed by the experiments of A. G. Jacques (75) on the penetration of  $H_2S$  into *Valonia* (see also 9, 69, 76).

In the case of weak acids it is usually assumed in the literature that the undissociated molecules penetrate more rapidly. This is to be expected on the basis of what has just been stated; for a fuller discussion see p. 303.

It is possible that this treatment can also be applied when  $HA$  is a strong acid, for if  $H^+$  and  $A^-$  collide at the surface they may form a molecule which can pass into the non-aqueous layer just as when  $HCl$  passes from water into air. On this basis the rate of entrance would be proportional to the number of collisions<sup>2</sup> and hence to the product  $(H)(A)$ . It follows that in the case of a strong acid (with no dissociation in the non-aqueous layer) multiplying  $H^+$  and  $A^-$  by two would multiply the product  $(H)(A)$  and, consequently, the rate of entrance by four.<sup>3</sup> This could not happen in the absence of a non-aqueous layer. This conception has not received an adequate test but there is evidence that strong acids enter more rapidly at low pH (45, 46, 48, 127). Regarding the entrance of  $HNO_3$  see 126.

Since the rate of entrance appears to depend chiefly on the two non-aqueous layers (99, 1013)<sup>4</sup> which may, for convenience, be treated as a single layer (84), we may assume that the rate of entrance of acids will be proportional to  $(H_o)(A_o) - (H_i)(A_i)$

<sup>2</sup> We arrive at the same result by assuming, for purposes of calculation, that a few molecules of  $HA$  exist in the external solution.

<sup>3</sup> This would cause the partition coefficient to increase with concentration. This has been observed in the case of models (97, 400; 116, 117) but varies in different compounds. It is affected by the degree of dissociation in the non-aqueous phase. In the case of  $KCl$  distributed between water and guaiacol the increase in the partition coefficient is much less than in the case of potassium guaiacolate.

<sup>4</sup> An analogous situation is found in certain models (p. 297) in which the protoplasm is represented by a layer of guaiacol which is vigorously stirred but which has unstirred layers at each of its surfaces. The unstirred layers correspond to the non-aqueous surface layers of the protoplasm and the intermediate stirred portion corresponds to the aqueous layer of the protoplasm where the transport of many substances (e.g., salts) is presumably much more rapid than in the non-aqueous layers; this is apparently due to their low partition coefficients (p. 301) rather than to diffusion constants (cf. 84).

where the subscripts *o* and *i* refer to outside and inside. Since the chemical potential is proportional to the product  $(H)(A)$ , the rate will depend on the difference in chemical potential, *i.e.*, on the driving force (when there is dissociation in the non-aqueous layer, this will be less exact and in any case it is only an approximation).<sup>5</sup>

*Bases.* In the case of bases the concentration gradients in the protoplasm appear to depend on chemical combination. As an illustration, we may consider the penetration of  $\text{NH}_3$  into *Valonia* (100).<sup>6</sup>

When we add  $\text{NH}_4\text{Cl}$  to the sea water it soon makes its appearance in the sap, entering chiefly as  $\text{NH}_3$ <sup>7</sup> (or  $\text{NH}_4\text{OH}$ ). If  $\text{NH}_3$  penetrated by simple diffusion,<sup>8</sup> the rate of entrance would be proportional to  $\text{NH}_{3o} - \text{NH}_{3i}$  (where the subscripts *o* and *i* refer to concentrations outside and inside, respectively). This is not the case. As  $\text{NH}_{3o} - \text{NH}_{3i}$  increases, the rate fails to keep pace with it but appears to approach a limit (Fig. 1).

How can this be accounted for? A simple explanation, suggested by the study of models (p. 297; also 99, 992) is that  $\text{NH}_4\text{OH}$  combines with a constituent  $\text{HX}$  of the protoplasm according to the equation  $\text{NH}_4\text{OH} + \text{HX} \rightleftharpoons \text{NH}_4\text{X} + \text{H}_2\text{O}$ . Then  $\text{NH}_4\text{X}$  diffuses inward,<sup>9</sup> so that the rate of entrance depends on the concentration of  $\text{NH}_4\text{X}$  in the protoplasm rather than on that of  $\text{NH}_3$  in the external solution.

The relative amount of  $\text{NH}_4\text{X}$  found at the outer surface of the protoplasm at each concentration of  $\text{NH}_{3o}$  may be ascertained from the formula

$$(\text{NH}_{3o}) (\text{HX}_b - \text{NH}_4\text{X}_e) = k (\text{NH}_4\text{X}_e)$$

where *k* is a constant and the subscripts *b* and *e* refer to molar concentrations at the beginning and when the reaction has reached

<sup>5</sup> There are complicating factors (109) and, as explained later, the rate will be proportional also to the permeability of the protoplasm which depends on its chemical composition and on its structure.

<sup>6</sup> Regarding the penetration of weak electrolytes into these cells see, 13, 31, 54, 55, 57, 58, 63, 69.

<sup>7</sup> This is shown by adding .001 M  $\text{NH}_4\text{Cl}$  to sea water and changing the pH. As the pH is lowered the rate of entrance falls off very rapidly (100).

<sup>8</sup> Regarding laws of diffusion see 71, 122, 123, 125.

<sup>9</sup>  $\text{NH}_4\text{X}$  is presumably formed in the outer non-aqueous layer of the protoplasm and diffuses through this layer. It may not be very soluble in water and may pass through the aqueous layer of the protoplasm chiefly in some other form (*e.g.*, as  $\text{NH}_3$  or as  $\text{NH}_4\text{Z}$ ) but this will be left out of account in the present discussion.

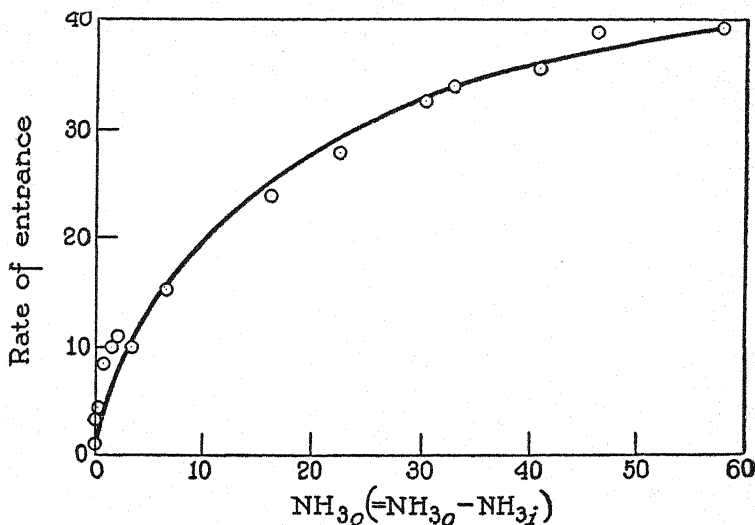


FIG. 1. Graph indicating that  $\text{NH}_3$  enters *Valonia* by combining chemically with the protoplasm. The rates of entrance (moles of  $\text{NH}_3 + \text{NH}_4$  entering 1 liter of cells in 10 minutes) are plotted as ordinates and the values of  $\text{NH}_3_o = \text{NH}_3_o - \text{NH}_3_i$  as abscissae, where the subscripts *o* and *i* refer to concentrations outside and inside, respectively; circles show observed values. See 100.

It is assumed that  $\text{NH}_3$  enters by combining with a constituent of the protoplasm,  $\text{HX}$ , to form  $\text{NH}_4\text{X}$ , and that the rate of entrance is directly proportional to the concentration gradient of  $\text{NH}_4\text{X}$ . The rate of entrance is taken as proportional to the concentration of  $\text{NH}_4\text{X}$  at the outer surface since in such brief experiments its concentration at the inner surface is negligible.

The curve shows the rate of entrance calculated on the basis of the reaction  $\text{NH}_3 + \text{HX} \rightleftharpoons \text{NH}_4\text{X}$  from the formula  $(\text{NH}_3)(\text{HX}_b - \text{NH}_4\text{X}_e) = .001533 (\text{NH}_4\text{X}_e)$  where the subscripts *b* and *e* refer to the beginning of the reaction and to equilibrium, respectively ( $\text{HX}_b$  is taken as .005 M).

The value of  $\text{NH}_3_i$  is taken as zero because the experiments last only 10 minutes and most of the  $\text{NH}_3$  which enters during this time is changed to  $\text{NH}_4^+$  on account of the low pH of the sap.

equilibrium, respectively. The curve in Fig. 1 is obtained by putting  $k = .001533$  and  $\text{HX}_b = .005$  M.

On reaching the sap,  $\text{NH}_4\text{X}$  may react with  $\text{HCl}$  to form  $\text{NH}_4\text{Cl}$ , or with  $\text{CO}_2$  to form  $\text{NH}_4\text{HCO}_3$  after which  $\text{HCO}_3^-$  may be exchanged for  $\text{Cl}^-$  coming in from the outside: we suppose that  $\text{HCO}_3^-$  and  $\text{Cl}^-$  move chiefly in molecular form, i.e., as  $\text{CO}_2$  and  $\text{HCl}$ , through the non-aqueous surface layer of the protoplasm since these layers, being immiscible with water, must have low dielectric constants which permit little dissociation (116).



The strong base guanidine (74) gives a curve resembling that in Fig. 1.<sup>10</sup> Perhaps this applies also to lithium whose entrance is, according to Collander (28a), relatively more rapid in dilute solutions. Although similar experiments cannot be carried out with potassium (because it penetrates very slowly and is already present in high concentration in the sap) there is evidence that it enters *Valonia* chiefly as KOH (99, 985; also 77, 79). We find that unless the ionic activity product  $[K][OH]$  is greater outside than inside, potassium does not enter the cell, and it penetrates faster as the product increases. When this product becomes greater inside (owing to an increase in  $OH_4$ ) potassium leaves the cell, although sodium continues to enter because the product  $[Na][OH]$  remains greater outside (77). This is shown in Fig. 2. In *Nitella* no such effect of external pH has been detected (80).

On this basis we should say that potassium enters because the chemical potential of KOH is greater outside than inside. If we assume that in the sap  $KX$  reacts with  $CO_2$  to form  $KHCO_3$  and that  $HCO_3^-$  is exchanged for  $Cl^-$  coming in from outside, we have a possible picture of the process (99, 983). (We suppose that  $HCO_3^-$  and  $Cl^-$  move mostly in molecular form through the non-aqueous surface layer whose low dielectric constant permits little dissociation.)

If the exchange of  $HCO_3^-$  or other organic anions were not complete we should find cations in the cell paired with organic anions to a considerable extent. This has actually been demonstrated (72, 73) in flowering plants.

When bases enter as hydroxides we may expect certain relations among which are the following:

(1) Increased production of carbon dioxide (and other organic acids) will promote growth and the absorption of electrolytes:<sup>11</sup> the reasons for this are clearly seen in models (99, 1002; 81, 83, 108). (It is commonly said that the energy needed for accumulation is due to respiration but in the model we can bring about accumulation without deriving any energy from the formation of carbon dioxide. We merely employ it after it has been produced

<sup>10</sup> If the partition coefficient increased rapidly enough with concentration<sup>3</sup> the first part of the curve might be convex to the horizontal axis. Our present data do not permit us to decide whether this occurs.

<sup>11</sup> I.e., by favoring the entrance of bases and by providing anions for exchange with inorganic anions in the external solution.

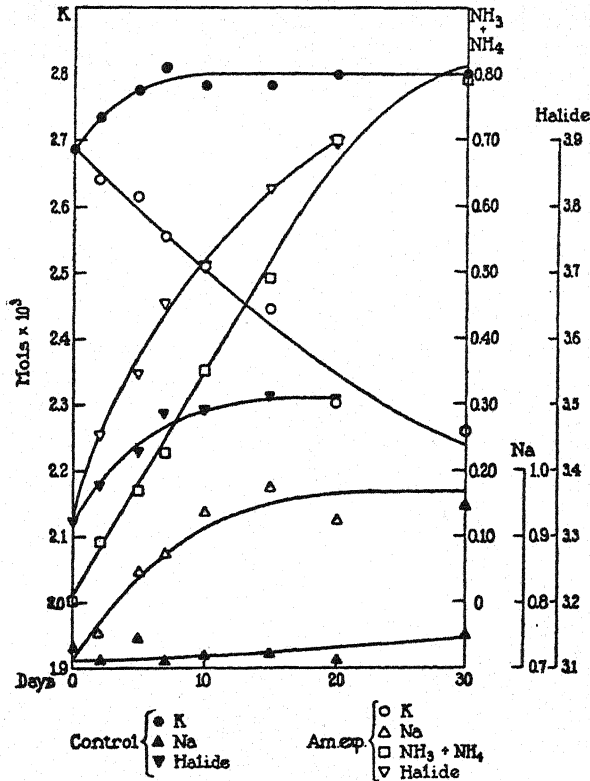


FIG. 2. Graphs showing the change in moles of K, Na,  $\text{NH}_3 + \text{NH}_4$ , and halide in a typical lot of cells of *Valonia macrophysa* in sea water (control) and in an equivalent lot of cells in sea water containing .001 M  $\text{NH}_4\text{Cl}$  (the scales of ordinates for each substance are the same but are displaced vertically to bring the curves into one figure). The entrance of  $\text{NH}_3$  is accompanied by a rise in the pH value of the sap (not shown here) which causes K to come out while Na continues to go in. It should be noted that the ordinates do not refer to concentrations but to the total number of moles in a certain lot of growing cells. The growth is proportional to the increase in moles of Cl in the control (where the concentration of Cl remains nearly constant) and this is approximately true for the cells exposed to  $\text{NH}_4\text{Cl}$  (where a small increase in the concentration of Cl occurs). (77).

elsewhere, thus using what is usually regarded as a waste product of the cell.)

This is quite in harmony with the fact that, in general, the greatest growth and absorption of electrolytes are found when there is the greatest production of carbon dioxide, as in germinating seeds, in flower formation and at growing points generally (99, 984; see also 42, 43, 86).

Steward (119, 120) and Steward and Berry (121) state that absorption of electrolytes increases as respiration increases but not necessarily in direct proportion. This is to be expected for if potassium enters as KOH, the value of  $(K_o)(OH_o) - (K_i)(OH_i)$  will not necessarily be doubled by doubling the rate of respiration. They think that other kinds of vital activity also play a rôle. Other investigators find a relation between respiration and absorption (44, 86, 87, 114, 114a).

(2) Absorption of cations and growth would be favored by a rise in external pH within certain limits.<sup>12</sup> The limits will depend on the nature of the case. Beginning at the lowest pH at which the cell can grow, we should expect the growth to increase with increasing pH until secondary changes<sup>13</sup> occur which have an inhibiting effect. This seems to be generally the case (79). Hence there is an optimum pH.

*Salts.* Evidently a salt like  $NH_4Cl$  might enter as such or might combine to form  $NH_4X$ , but if such combination occurs it must be very restricted for  $NH_4Cl$  enters very slowly as compared with  $NH_3$ .<sup>14</sup>

A similar state of affairs appears to exist in certain models (p. 296), in which the protoplasmic surface is represented by the organic acid guaiacol (which may be called HG). Here  $NH_3$  enters by forming  $NH_4G$  which on reaching the "artificial sap" reacts with  $CO_2$  to form  $NH_4HCO_3$ ; this applies also to potassium. We find that  $NH_3$  and KOH enter rapidly<sup>15</sup> but  $NH_4Cl$  and KCl penetrate very slowly, as with *Valonia*. However, if KCl enters *Valonia* as such, it does so against a gradient; this applies also to  $NH_4Cl$  when its concentration is higher inside, but it enters very slowly (if at all) as  $NH_4Cl$  even when it goes with the gradient.

The anion of the salt may enter together with  $H^+$  which will make the external solution more alkaline (126). See p. 303.

<sup>12</sup> This takes place when green cells are illuminated (99, 986) and may explain the favorable effect of light on absorption in some cases.

<sup>13</sup> A possible example of such changes is seen in *Nitella* when exposure to alkaline solutions causes great changes in the properties of the protoplasmic surface (104).

<sup>14</sup> For the rapid penetration of  $NH_3$  (i.e., of  $NH_4X$ ) as compared with  $NH_4Cl$  see 100. The more rapid penetration of KOH as compared with KCl is indicated by the fact that when the external pH rises, potassium enters more rapidly although the external concentration of KCl remains the same (79).

<sup>15</sup> Regarding acetic acid and potassium acetate see p. 303.

*Rôle of ionic transfer.* These experiments indicate that electrolytes pass through the non-aqueous protoplasmic surface chiefly in molecular form since its low dielectric constant would not permit much dissociation. That some dissociation takes place and that ions can enter to some extent is shown by the bioelectrical effects (99, 997), but the concentration of ions is very small, as shown by the high electrical resistance and impedance of the surface (p. 284; also 99, 1012; 24, 25, 26, 27, 35, 37, 38). Owing to the low dielectric constant of the protoplasmic surface, all electrolytes will be weak in the surface layer (116) and the transport of ions through the surface must play a very subordinate rôle (99, 994, 1004).

This applies whether we suppose the ions to enter as dissociated molecules (anions and cations entering together) or by ionic exchange (*e.g.*,  $K^+$  entering in exchange for  $H^+$  coming out) as assumed by some investigators (50; 68; 99, 994). For equations for the exchange of ions see 95.

With *Valonia* it is possible (by making certain assumptions) to calculate the rate of entrance by ionic exchange as compared with that on the basis described above and in every case the result is adverse to the idea of ionic exchange (99, 994; 78).

If the entrance of electrolytes depended chiefly on ionic transport, it should be possible to predict quantitatively the relative rates of entrance from the apparent mobilities of the ions in the protoplasm, but this is not possible (99, 1006).<sup>16</sup> For example, on the basis of ionic mobilities we should predict that cesium would enter about as rapidly as sodium,<sup>17</sup> but it penetrates very much more slowly (30).

In view of this, it is not surprising that our experiments yield evidence against the suggestion of Michaelis (99, 990) and of Höber that the entrance of electrolytes is determined by the charge on the surface of the protoplasm. For example, in *Valonia* this concept meets with difficulties, since with KCl we should on this basis have to say that the surface is negatively charged (as the more dilute solution is electrically positive in the external circuit). But, as Damon has shown, with NaCl we should have to conclude

<sup>16</sup> Regarding the penetration of strong electrolytes see (14, 15, 17, 18, 19, 22, 30).

<sup>17</sup> Damon (unpublished results) finds that the apparent mobility of  $Cs^+$  in *Valonia* does not differ greatly from that of  $Na^+$ .

that the surface has a positive charge, since the more dilute solution is negative (32).

This difficulty can be avoided by ignoring the effect of any possible charge on the surface. We can then calculate the apparent relative mobilities of  $K^+$ ,  $Cl^-$  and  $Na^+$  in the non-aqueous surface layer to be 20, 1 and .2, respectively. Damon finds (unpublished results) that the order of mobilities is  $Rb > Cl > Na$ ; this is the order found in water and it seems reasonable for a non-aqueous layer. This would account for the electrical behavior on the basis of diffusion potential without any assumption regarding a charge on the surface.

If the surface charge determined the entrance of electrolytes it is difficult to see how both anions and cations could enter in equal numbers, as happens in *Valonia*; for a negative charge would inhibit the entrance of anions and a positive charge that of cations. There is no difficulty, however, when we ignore the effect of charge and suppose the protoplasmic surface to act like a non-aqueous layer, e.g., guaiacol plus quinoline, through which  $K^+$  and  $Cl^-$  pass in equal numbers (unpublished results).

The views here presented are at variance with those of several authors who maintain that electrolytes enter chiefly by ionic exchange. Regarding these, see 8, 8a, 8b, 15, 17, 21, 38a, 50, 51, 68, 90, 91.

*Permeability of the protoplasm.* The rate of entrance depends not only on concentration gradients in the protoplasm but also on the permeability of the protoplasm which may be defined for HA as the number of moles entering in unit time under standard conditions<sup>18</sup> when the value of  $(H_o)(A_o) - (H_i)(A_i)$  is unity. This definition arises from the conception that the rate of entrance is proportional to  $(H_o)(A_o) - (H_i)(A_i)$  and cannot be more than an approximation (p. 285). It applies to weak acids but not to Fig. 1 except where the curve approximates a straight line. It is, nevertheless, useful when its limitations are recognized.

Owing to variability<sup>19</sup> of the protoplasm, the safest method is to make all measurements comparative, using the same substance as a control in every case.

<sup>18</sup> I.e., of temperature, area, etc. (Cf. 99, 990).

<sup>19</sup> This depends on both its chemical composition (cf. 60) and its structure (p. 293). For methods of determining permeability see 99, 979; 68, 69, 69a, 70, 70a, 71, 71a, 84a, 84b.

*Nature of the time curve.* Before concluding this section it is desirable to call attention to changes of concentration with time. In *Valonia* and *Nitella* the time curve for entrance of certain dyes (11, 53) and of bromide (49) is of the first order. This applies also to the exit of dyes (56). In some cases experiments indicate the second order for *Valonia* (10, 75, 76) but this might result from variability in the permeability of the cells. For if the time curve were really of the first order and in some cells (*e.g.*, in those of smaller size or greater permeability) penetration were completed more rapidly, the process would appear to proceed more rapidly at first and then slow down when the average of all the cells was taken (99, 998).

In these brief experiments there is little or no entrance of water; for equations see 93, 95. When water enters, the situation changes and for equations dealing with this see 82, 83, 84, 98; also 70.

*Temperature coefficient.* The temperature coefficient is, in general, high. This does not necessarily indicate a chemical reaction though this seems to occur in certain cases (p. 286). For dyes, Irwin found  $Q_{10} = 4$  or more (53, 56); Hoagland, Hibbard and Davis (49) found  $Q_{10}$  between 2 and 3 for the entrance of bromide. Irwin found  $Q_{10} = 2.3$  in a chloroform model (66).<sup>20</sup>

Some very important aspects of kinetics can be more conveniently treated in later sections, *e.g.*, partition coefficients (p. 301); steady state (p. 296).

*Influence of one substance on another.* The diffusion constant and the partition coefficient of any substance in the non-aqueous layer may be affected by the presence of other substances.

A variety of factors may affect the rate of penetration (79, 81, 82, 83, 84, 107, 109, 110, 111) and when the entering electrolyte enters into chemical combination with the protoplasm (100), one substance may affect the entrance of another by competing for the substance which acts as a carrier (99, 999) if the latter is limited in amount. An influence might be exercised (99, 999) by any substance which alters the viscosity, thickness or chemical composition of the protoplasmic surface, as seen, for example, in antagonistic action or in the effects of distilled water (106) or in changes in the pH of the protoplasm or of its surroundings (99, 999; 79).

Of especial interest are the other variables which have been dealt

<sup>20</sup> For recent theoretical considerations see 1, 33.

with by Irwin (54, 55), McCutcheon and Lucké (88), Hoagland, Davis and Hibbard (45, 47, 48). When the absorption of potassium changes as the external concentration of calcium increases (85), the result may depend on physiological balance which affects the state of the surface (*cf.* 99, 999).

Very striking results were found by Irwin (60). Treatment of *Nitella* for a short time with .01 M NaCl<sup>21</sup> reduced the rate of penetration, when the cells were subsequently exposed to cresyl blue, by about 50 per cent. This effect of NaCl could be removed by rinsing the cells in a solution of MgCl<sub>2</sub> or CaCl<sub>2</sub> before exposing to the dye. In models, the addition of salicylic acid greatly reduced the penetration of cresyl blue but not of phenol red (66).

#### ACCUMULATION

*Nature of accumulation.* It is customary to speak of accumulation when, for example, the concentration of K<sup>+</sup> becomes higher inside than outside and it is often implied that energy is required to bring this about. But we know that if a system is moving toward a Donnan equilibrium (*i.e.*, with a rundown of energy) potassium may be entering and reaching a much higher concentration inside than outside.

It would seem logical to reserve the term "accumulation" for cases where an expenditure of energy is required, *i.e.*, where the chemical potential of a compound rises to a higher level inside than outside. In that case, we might speak of the accumulation of KCl but refrain from speaking of the accumulation of K<sup>+</sup> as being ambiguous from the standpoint of thermodynamics.

The fact that K<sup>+</sup> can reach a much higher concentration inside than outside is evident from Tables I and II. At one time it seemed natural to try to account for such cases by means of the Donnan equilibrium but this is impossible in the case of the cells just referred to.

In the first place, the chemical potential of KCl, which is proportional to the ionic activity product [K] [Cl], is, in most cases, much higher inside the cell than outside (Table II) (in the Donnan equilibrium it is equal on both sides).

In the second place, the ratios of the various ions inside to those

<sup>21</sup> This effect is apparently not produced by treatment with distilled water.

TABLE I  
Chemical Analyses of Sap (The numbers in parentheses denote percentage when Cl is taken as 100)

|                                | Sea water <sup>1</sup> | Sap of <i>Valonia macrophylla</i> <sup>1</sup> (Bermuda) | Sap of <i>Valonia ventricosa</i> <sup>2</sup> (Florida) | Sap of <i>Halcyotis Osterhoutii</i> <sup>3</sup> (Bermuda) | Sap of <i>Nitella clavata</i> <sup>4</sup> (California) | Pond water bathing <i>Nitella clavata</i> <sup>4</sup> | Sap of <i>Chara ceratophylla</i> <sup>5</sup> (Finland) | Brackish water bathing <i>Chara ceratophylla</i> <sup>5</sup> |
|--------------------------------|------------------------|--|---|--|---|--|---|---|
| Cl*                            | M<br>0.580<br>(100.00) | M<br>0.597<br>(100.00)                                   | M<br>0.608<br>(100.00)                                  | M<br>0.603<br>(100.00)                                     | M × 10 <sup>3</sup><br>90.8<br>(100.00)                 | M × 10 <sup>3</sup><br>0.903<br>(100.00)               | M × 10 <sup>3</sup><br>225.0<br>(100.00)                | M × 10 <sup>3</sup><br>73.0<br>(100.00)                       |
| Na                             | 0.498<br>(85.87)       | 0.09<br>(15.08)  | 0.0348<br>(5.73)  | 0.557<br>(92.4)  | 10.0<br>(11.0)  | 0.217<br>(24.0)  | 142.0<br>(63.1)   | 60.0<br>(82.2)  |
| K                              | 0.012<br>(2.15)        | 0.5<br>(86.24)   | 0.576<br>(94.74)  | 0.0064<br>(1.01)   | 54.3<br>(59.8)  | 0.051<br>(5.6)   | 88.0<br>(39.1)  | 1.4<br>(1.9)  |
| Ca                             | 0.012<br>(2.05)        | 0.0017<br>(0.285)  | Trace   | 0.008<br>(1.33)  | 10.2<br>(11.2)  | 0.775<br>(85.8)  | 5.3<br>(2.4)  | 1.8<br>(2.5)  |
| Mg                             | 0.057<br>(9.74)        | Trace?   | Trace   | 0.0167<br>(2.77)   | 17.7<br>(19.5)  | 1.69<br>(187.0)  | 15.5<br>(6.9)   | 6.5<br>(8.9)  |
| SO <sub>4</sub>                | 0.036<br>(6.26)        | Trace?   | Trace   | Trace  | 8.33<br>(9.2)   | 0.323<br>(35.8)  | 3.9<br>(1.8)  | 2.8<br>(3.9)  |
| H <sub>2</sub> PO <sub>4</sub> | —                      | —  | —   | —  | 3.61<br>(4.0)   | 0.0002<br>(0.02)                                       | 4.1<br>(1.8)  | Trace   |
| NO <sub>3</sub>                | —                      | —  | —   | —  | 0<br>(0)  | 0.55<br>(60.8)   | 0.4<br>(0.18)   | 0.005<br>(0.007)  |
| pH**                           |                        |  |   |  |   |  |   |   |
|                                | 8.                     | 5.8  | 5.6-6.0†  | 5  | 5.2   | 7.2  | 5.9††   | 7.9   |

For analyses of *Valonia* at Naples (which are not in complete agreement) see 89, 39, 112, 113, 20. S. C. Brooks (16) finds in *Halcyotis ovalis* (Lyng.) Areschoug of the Pacific coast the ratio  $K \div Na = 1.5$ ; see also 52. S. C. Brooks (20) calls attention to differences in the  $K \div Na$  ratio of different species in different localities. It should be remembered that there may be considerable difference in the same species in the same locality. Thus, in Bermuda the  $K \div Na$  ratio in *Valonia macrophylla* Kütz varies from 2.55 to 5.72 (77; 97, 370). The sap of *Valonia macrophylla* contains 1.4 parts per thousand of organic matter and that of *Chara ceratophylla* less than 30 parts per thousand (28).

<sup>1</sup>Cf. 92, where "parts per thousand" of Ca in the sap, as given, is 10 times too large. See also 15. <sup>2</sup>Cooper and Blinks (29). The figures are here corrected for slight evaporation. This species is presumably *V. ventricosa* J. G. Ag. <sup>3</sup>Blinks and Jacques (7) (the figure for total gram equivalents of cations should be given as 0.613). Blinks (2). <sup>4</sup>Zscheile (128), figures revised from Hoagland and Davis (45) which see for other analyses; also (47). <sup>5</sup>Collander (28).

\*Including Br calculated as Cl. \*\*Except in the case of sea water the values given represent a rough general average; the determinations are doubtful because the sap is but slightly buffered. †M. M. Brooks (12); M. Irwin (unpublished results). ††No allowance for salt error.



TABLE II  
Ratio of Internal to External Concentration  
(Conc. in sap ÷ conc. in surrounding medium)

|                                | <i>Valonia<br/>macro-<br/>physa</i><br>(Bermuda) | <i>Valonia<br/>ventricosa</i><br>(Florida) | <i>Halicystis<br/>Osterhoutii</i><br>(Bermuda) | <i>Nitella<br/>clavata</i><br>(Cali-<br>fornia) | <i>Chara<br/>cerato-<br/>phylla</i><br>(Finland) |
|--------------------------------|--|--|--|---|--|
| Cl ....                        | 1.03   | 1.05                                       | 1.04   | 100.50  | 3.1  |
| Na ...                         | 0.18   | 0.07                                       | 1.12   | 46.10   | 2.4  |
| K ....                         | 41.6   | 48.0                                       | 0.53   | 1065.00   | 63.0   |
| Ca ...                         | Very small                                       | Very small                                 | 0.67   | 13.17   | 2.9  |
| Mg ..                          | Very small                                       | Very small                                 | 0.29   | 10.47   | 2.4  |
| SO <sub>4</sub> ..             | 0  | 0  | 0  | 25.80   | 1.4  |
| H <sub>2</sub> PO <sub>4</sub> | —  | —  | —  | 18050.00  | > 400.0  |
| NO <sub>3</sub> ..             | —  | —  | —  | 0   | 80.0   |
| H* ...                         | 158  | 158  | 1000   | 100   | 100  |

\* The values increase when photosynthesis raises the pH just outside the protoplasmic surface.

outside do not in the least correspond to the Donnan equilibrium (Table II).

In the third place, we find in the case of *Valonia macrophysa*, for example, no indiffusible ions in sufficient concentration to bring about such great differences. It is true that Donnan ratios might occur in the absence of indiffusible ions, as pointed out by Teorell (122, 123, 125), given a sufficient potential due to outward diffusion of an electrolyte. Evidently this does not happen in the cells here considered for they show no Donnan ratios, as is evident from Table II (99, 981).

Moreover, equilibrium is impossible as long as metabolism and growth continue. In place of an equilibrium we have in these cells a steady state. So long as they are growing, water and electrolytes enter in a fixed ratio so that the composition of the sap remains much the same while its volume increases. Hence the substance which penetrates most rapidly is the one which predominates in the sap (99, 980).

Internal concentration probably depends, in many cases, on the fact that the cellulose wall restricts the entrance of water, thus favoring increase in concentration of electrolytes inside. As the cell matures, growth and the production of carbon dioxide fall off and changes in permeability may tend to prevent the egress of sub-

stances already accumulated; perhaps this happens in the case of human erythrocytes which appear to allow no potassium to pass out. Regarding models of mature cells see 81.

*Accumulation in models.* Accumulation occurs in models (modified from those described by Irwin) consisting of an aqueous solution *C* representing the sap, a non-aqueous layer *B* representing the protoplasm, and an external aqueous solution *A* (Fig. 3). *A*

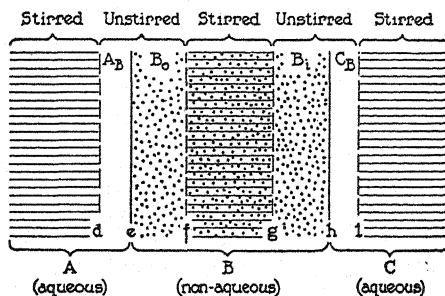


FIG. 3. Diagram of layers in the model. The aqueous phase *A* has an unstirred layer which is represented between *d* and *e*; from *e* to *f* is the corresponding unstirred layer in the non-aqueous phase *B*. Similar layers are present at the boundary between the non-aqueous phase *B* and the aqueous phase *C*.

contains KCl and is more alkaline than the sap (as is the case in *Valonia* and many other living cells); *B* consists of guaiacol + *p*-cresol (called G. C. mixture); for convenience we call them, collectively, HG. *C* contains, at the start, distilled water through which bubbles  $\text{CO}_2$  to imitate its production by the living cell.

Potassium passes through the non-aqueous layer and enters the artificial sap where its concentration becomes much higher than in the external solution. It first combines at the outer surface according to the equation  $\text{KOH} + \text{HG} \rightleftharpoons \text{KG} + \text{H}_2\text{O}$ . On reaching the artificial sap we have the reaction  $\text{KG} + \text{H}_2\text{CO}_3 \rightleftharpoons \text{KHCO}_3 + \text{HG}$ . In time, a steady state is set up in which water and electrolyte enter in a fixed ratio so that the volume of the "artificial sap" increases while its composition stays nearly constant (this seems to be similar to what happens in living cells).

Evidently HG remains constant in amount and merely acts as a carrier. From a thermodynamic standpoint we have an exchange of  $\text{K}^+$  in the external solution for  $\text{H}^+$  in the artificial sap. But from a kinetic standpoint such a picture is entirely misleading, for

when KG moves through the non-aqueous layer it does so chiefly in molecular form, as the dielectric constant is too low to permit much dissociation. According to Shedlovsky and Uhlig (116), the dissociation constant of KG in guaiacol is about  $5 \times 10^{-5}$ .

This seems to resemble what happens in *Valonia*. The models furnish other important analogies (81, 98, 107, 108, 109, 110, 111). In the model, as in *Valonia*, the order of penetration\* is  $K > Na > Mg > Ca$ ; similar differences between the external and internal solutions are found in both in respect to pH, free energy, etc. (cf. 111). Similarities exist in respect to ionic mobilities (116) and potential (102a). Furthermore, bases enter by combining but this does not apply to acids.

We may say that when the internal activity product  $[K][Cl]$  becomes greater inside a living cell than outside, KCl accumulates and energy is required for this process. This must be furnished by chemical activity.<sup>22</sup> Actually, the internal product  $[K][Cl]$  is greater than the external in *Valonia* (Table I), in *Nitella* (45, 47), in *Chara* (28) and in many other living cells (72, 73).

The models likewise have chemical activity which can make the product  $[K][Cl]$  greater inside than outside. To show that KCl can penetrate we place distilled water in C (with no CO<sub>2</sub> bubbling) and .1 M LiCl and .05 M KOH or .05 M KG in A. We find that these substances pass into C so that in the course of time A and C would become identical in composition. To hasten this we empty C and fill it with solution taken from A. We then start CO<sub>2</sub> bubbling in C and presently the concentration of potassium begins to increase; this continues until it becomes about four times as great as in A. The ionic concentration product (K) (Cl) is then about four times as great in C as in A. The ionic activity product  $[K][Cl]$  is a little less because the activity coefficients are somewhat smaller in C than in A owing to the difference in ionic strength (108).

The energy necessary for this marked rise in the chemical potential of KCl (which is proportional to the product  $[K][Cl]$ ) in C

\* This is the order of penetration through the body wall of a holothurian (81a, 81b, 81c, 81d).

<sup>22</sup> In the model, K<sup>+</sup> can reach a much higher concentration inside than outside without raising the chemical potential of KCl above that outside. This is because K<sup>+</sup> enters much more rapidly than Cl<sup>-</sup> so that, for example, when K<sup>+</sup> is four times as concentrated inside Cl<sup>-</sup> is less than one-fourth as concentrated in which case the product  $[K][Cl]$  is less than outside.

is derived from the reactions occurring in the system and from the continual renewal of the solution in  $A$  and from the supply of  $\text{CO}_2$ .

Evidently the system has sufficient energy to raise the chemical potential of  $\text{KCl}$  to a higher level inside than outside just as the living cell does. In the cell both  $\text{K}^+$  and  $\text{Cl}^-$  can reach a higher concentration inside.<sup>23</sup> But in the model  $\text{Cl}^-$  does not behave thus. In this respect it differs from  $\text{K}^+$ , for  $\text{K}^+$  enters as  $\text{KG}$  which moves rapidly through  $B$  but passes out as  $\text{KHCO}_3$  which moves slowly. The cell apparently has a device for making  $\text{Cl}^-$  move out more slowly than it moves in. Our imitation would be more complete if we could introduce this into the model, *e.g.*, by finding a substance which takes up more  $\text{KCl}$  as the pH rises (for the rôle of partition coefficients see p. 301). If, for example, a substance were employed that takes up more water at higher pH, it might take up more  $\text{KCl}$ , since it often happens that the more water a non-aqueous substance contains the more inorganic electrolyte it will take up from an aqueous solution.

The fact that accumulation depends on a relatively rapid inward movement has been emphasized by Irwin who shows that it is aided by the difference in partition coefficients at the inner and outer protoplasmic surfaces (61, 62, 64, 65, 67).

It is easy to make a model in which  $\text{K}^+$  goes in against a concentration gradient or a different model in which  $\text{Cl}^-$  goes in against a concentration gradient but whether both these things can happen in the same model remains to be seen.

Since the cell has energy at its disposal, it is not surprising that  $\text{KCl}$  can accumulate even when no potassium compound seems to have an excess of chemical potential outside. This appears to happen in *Nitella* under certain conditions (80, 100). In this respect *Nitella* differs from *Valonia* where  $\text{KOH}$  has an excess of chemical potential outside. But *Nitella* requires no more energy to produce a given excess of chemical potential of  $\text{KCl}$  inside than does *Valonia*. The chief difference is that in the case of *Valonia* the first step in the process may be the entrance of  $\text{KOH}$ , but there is no evidence of this in *Nitella*. The energy necessary for accumulation is derived from metabolism and it has been suggested by Steward and Berry (121) and by Hoagland and Broyer (44) that

<sup>23</sup> In *Valonia* the concentration of  $\text{Cl}^-$  is only slightly higher inside, *i.e.*, .6 M inside and .58 M outside. See Table I, p. 295. In *Nitella* the activity of  $\text{Cl}^-$  is much higher inside than outside.

certain kinds of metabolism are of especial importance in this connection.

Although the cell has a supply of energy by which it can raise chemical potentials (*e.g.*, that of KCl) to a higher level inside than outside and can cause both  $K^+$  and  $Cl^-$  to reach a higher concentration inside (Table I), the steps by which this is brought about need investigation. The cell does not use its energy to bring about such a result with all substances. In fact, with *Valonia* only potassium and ammonium compounds are normally so treated. This is because of their rapid penetration. It would seem that the sodium, magnesium and calcium compounds penetrate slowly as compared with water so that the internal chemical potential does not rise as high as it otherwise would. Cells which have ceased to grow might show a different situation but such cells may have a different metabolism and may possibly become less permeable to certain substances.

The ammonium compounds are of especial interest. Although the chemical potential of  $NH_4OH$  (or the internal product  $[NH_4][OH]$ ) did not in any case become greater in *Valonia* sap<sup>24</sup> than outside<sup>25</sup>, the product  $[NH_4][Cl]$  in some cases became more than 100 times as great inside (31, 77). To judge from the data of Irwin (55) a similar situation obtains in *Nitella* with brilliant cresyl blue for, although the chemical potential of the undissociated dye base does not become greater inside than outside, that of the dye chloride does, since both the dye cation and  $Cl^-$  reach much higher concentrations inside than outside.

It is significant that the energy of the cell is not applied to bring about an accumulation of non-electrolytes. This would seem to indicate that it acts electrically. This is also the case with the models hitherto studied.

It may be added that those who regard the entrance of electrolytes as due chiefly to ionic exchange may not subscribe to the views here set forth. Regarding this see 8, 8a, 8b, 15, 17, 21, 38a, 50, 51, 68, 90, 91.

Let us now turn to the important problem of selective permeability.

<sup>24</sup> Osterhout, W. J. V. and Cooper, W. C., Jr., unpublished results.

<sup>25</sup> This is true also of *Halicystis* (*cf.* 2). For *Nitella* see 54.

## SELECTIVE PERMEABILITY

Selective permeability is well illustrated by the large cells employed in these studies. For example, *Valonia macrophysa* takes up about forty times as much potassium as sodium from the sea water although sea water contains relatively little potassium. In order to clarify the problem let us consider the chief factors involved.

When the sap of a growing cell remains approximately constant in composition it is evident that water and electrolyte must enter in a fixed ratio so that a steady state obtains in which the composition of the sap depends on the rate of absorption of its various components.

As an example we may take the penetration of sodium and potassium on the assumption that they enter as hydroxides, as stated above (p. 288). As a first approximation we may neglect the effect of their chemical reactions with the protoplasm since if their behavior in this respect is similar it will not greatly affect the comparison, especially at lower concentrations (100). Hence we may write as an approximation

$$\frac{R_{\text{KOH}}}{R_{\text{NaOH}}} = \frac{P_{\text{KOH}} ([K_o] [\text{OH}_o] - [K_i] [\text{OH}_i])}{P_{\text{NaOH}} ([Na_o] [\text{OH}_o] - [Na_i] [\text{OH}_i])}$$

where  $R_{\text{KOH}}$  is the rate of entrance of KOH,  $P_{\text{KOH}}$  is the permeability of the protoplasm to KOH, and the subscripts *o* and *i* refer to activities outside and inside, respectively. Substituting numerical values, we obtain  $P_{\text{KOH}} \div P_{\text{NaOH}} = 331$  (99, 991); that is to say, the protoplasm is 331 times as permeable to KOH as to NaOH.

What causes this great difference? Presumably the rate of entrance depends chiefly on two factors, namely:

(1) The diffusion constants in the protoplasm. If the entering electrolyte forms a compound with a constituent of the protoplasm the diffusion constant of this compound must be taken into consideration.<sup>26</sup> It is evident that we can not expect sufficient difference in the diffusion constants to account for this result.

(2) The concentration gradients in the protoplasm. These depend on the partition coefficients.<sup>3</sup> This may be illustrated by the use of models (Fig. 4).

<sup>26</sup> The entering electrolyte may form one compound in the non-aqueous and another in the aqueous layer of the protoplasm.<sup>9</sup>

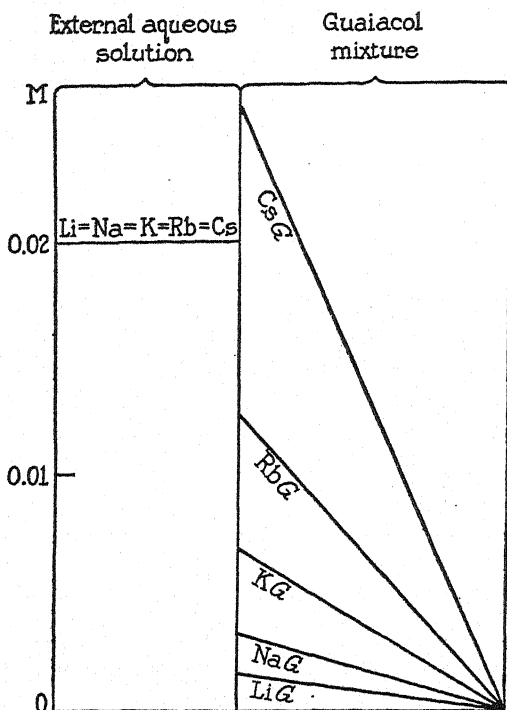


FIG. 4. Shows concentration gradients in the outer unstirred layer of the non-aqueous layer ( $B_o$  in Fig. 3) of the model (this is assumed to be thin enough to make the concentration gradients approximately linear).

In the external solution the concentrations are all .02 M and it is assumed that two salts are present in every case (*e.g.*, Na+K or Na+Cs, *cf.* 110). Evidently, when NaG and KG are present, the concentration gradient is about twice as great for KG as for NaG and it is found that the rate of entrance is correspondingly greater (110).

The order of penetration is that of ionic mobilities in water but as all these salts are very weak electrolytes in the guaiacol mixture they move through it chiefly in undissociated form. This is doubtless true of living cells.

When we place in the outside solution (*i.e.*, in *A*, Fig. 3) .02 M KOH + .02 M NaOH (or the corresponding concentration of KG and NaG) the concentrations are equal. But they are not equal in the non-aqueous layer *B* (in which diffusion is so slow that it controls the rate of entrance). Here the partition coefficient for KG is .35 and for NaG is .165 (110). Hence the concentration gradient across *B* at the start is  $.35(.02) = .007$  for potassium and  $.165(.02) = .0033$  for sodium. As the concentration gradient is

much greater for potassium than for sodium we might expect a much higher rate of entrance for potassium (84). This is actually the case (109, 110).

According to Shedlovsky and Uhlig (117), the partition coefficient is a function of the ionic radius and we should, therefore, expect the rate of entrance to increase in the order  $\text{Li} > \text{Na} > \text{K} > \text{Rb} > \text{Cs}$ , as is actually the case (110). Since it happens that this is also the order of ionic mobilities in water it might be considered a proof of entrance by ionic exchange did we not know that these are weak electrolytes in *B* (116) so that they move through it mostly in undissociated form (Fig. 4).

The partition coefficient of a compound will increase as the partition coefficients of the ions increase. Moreover, as the dielectric constant of the non-aqueous layer decreases the dissociation constant will follow suit and the proportion of undissociated molecules will become greater. With ammonia (p. 286) we have to do with  $\text{NH}_4\text{X}$ . If  $\text{X}^-$  is indiffusible we have the situation discussed by Teorell (125); this may not have much effect on the rate of entrance which depends chiefly on undissociated molecules.

Similar considerations doubtless apply to living cells for potassium usually enters more rapidly than sodium (but the difference may be much greater than is indicated by the equation of Shedlovsky and Uhlig and here chemical combination may play a rôle as already suggested on p. 286).

A similar dependence on partition coefficients appears when we compare the entrance of a weak acid and its salts. Thus the partition coefficient of molar acetic acid between water and guaiacol is about 30 times as large as that of molar potassium acetate.<sup>27</sup> Hence

<sup>27</sup> Unpublished results. This is to be expected on theoretical grounds. This may be illustrated by the following hypothetical case (the figures denote activities).

| Aqueous   | Non-aqueous  |
|---|--|
| $(\text{H}) (A) = k (\text{HA})$<br>$10^{-5} 10^{-4} = 10^{-6} 10^{-3}$     | $(\text{H}) (A) = k' (\text{HA})$<br>$10^{-4} 10^{-5} = 10^{-9} 10^{-1}$           |
| $(\text{Na}) (A) = k_s (\text{NaA})$<br>$9(10^{-5}) 10^{-4} = 1.9(10^{-9})$ | $(\text{Na}) (A) = k'_s (\text{NaA})$<br>$9(10^{-6}) 10^{-5} = 10^{-4} 9(10^{-7})$ |

Here we put the activity partition coefficient of the undissociated acid ( $\text{HA}$ ) and that of the undissociated salt ( $\text{NaA}$ ) the same, following the scheme of Lewis and Randall (Lewis, G. N., and Randall, M., *Thermodynamics*, New York, 1923, p. 262; see also Shedlovsky and Uhlig (116)).  $\text{NaA}$  is, of course, the stronger electrolyte in the non-aqueous.

If we now make the very reasonable assumption that the activity coefficients of undissociated  $\text{NaA}$  and of undissociated  $\text{HA}$  in the non-aqueous do not differ greatly the concentration of  $\text{NaA}$  in the non-aqueous will



the rate of penetration of the free acid is greater just as with living cells.

Regarding bases it may be said that in the models KOH penetrates more rapidly than KCl because KOH forms KG which has a higher partition coefficient than KCl and this appears to apply to *Valonia* (p. 290).

Regarding the penetration of dyes, see the papers of Irwin and of M. M. Brooks (bibliography in 99). Nirenstein (91a) and Irwin (66) found that both acid and basic dyes could enter simultaneously in certain models. It seems probable that the peculiarities of various cells may be imitated by means of models.

When substances are compared whose partition coefficients are similar, the order of penetration will be determined by their molecular weights. This seems to be the case with certain non-electrolytes (115, 118).

To recapitulate, we may say that selective permeability is determined chiefly by diffusion constants and concentration gradients and that the latter depend upon partition coefficients. The importance of partition coefficients was recognized by Overton but he considered only those at the outer phase boundary of the protoplasmic surface layer. Irwin has shown that the partition coefficients at the inner phase boundary of this layer must also be considered (64) (also papers cited in 99), and where a vacuole exists its surface layer must be taken into account (67).

The diffusion constants and partition coefficients are not constant but depend on temperature, concentration (110, 117), the presence of other substances, electrolytes and additional factors. Hence the permeability of the protoplasm varies.

With the aid of the facts set forth in the preceding sections we may now try to form a picture of the protoplasmic surface in these cells.

#### NATURE OF THE PROTOPLASMIC SURFACE

*It behaves as a liquid.* This is evident when protoplasm is squeezed out of these cells and comes into contact with water; it then rounds up like an oily liquid. According to Chambers (23), the surface of certain marine eggs also behaves as an oily liquid.

be very small and the concentration in the non-aqueous divided by that in the aqueous phase will be much greater in the case of  $HA$  than in that of  $NaA$  (and still more so if  $HA$  associates in the non-aqueous to form double molecules).

If the surface is liquid it cannot be a mosaic in the sense of Höber.<sup>28</sup>

In many cases, the protoplasm is normally covered with a layer of something analogous to the cellulose wall of plants (*e.g.*, chitin or cellulose) so that in experiments on cataphoresis or on wetting, we may be dealing with such materials rather than with the true protoplasmic surface. The protoplasm in contact with the vacuole of the cell is probably not covered in this way; its surface acts like an oily liquid at all times.

*It is non-aqueous.* As already stated, this is shown by a variety of evidence (99) and especially by electrical measurements. An example of the latter is seen in *Nitella*. A spot in contact with .01 M NaCl is 85 mv. positive to one in contact with .01 M KCl. This would not be possible with an aqueous gel, *e.g.*, protein imbibed with water (94).

The behavior of many substances, including weak acids and their salts,<sup>27</sup> would not be explainable on the basis of an aqueous surface (see p. 303).

*Its thickness.* It must be thick enough to account for the very slow inward diffusion of many substances which would enter rapidly in the absence of a non-aqueous layer. For example, salts enter very much more slowly than alcohol (*cf.* 99, 1010).

It seems doubtful whether a layer only one or two molecules thick could account for this situation. A layer of this thickness could not suffice in the case of rapid increase in cell surface nor of chemical combination between the surface and the entering electrolyte, which appears to happen in the case of penetrating bases (p. 286). Nor would it account for the great changes which can be experimentally produced in the surface layer. These will be considered more in detail in the next section.

*It is not homogeneous.* It cannot be homogeneous when profound modifications of the surface are possible which are reversible in character. For example, in *Nitella* one of the most striking properties of the protoplasmic surface is the ability to distinguish between sodium and potassium; in this respect it acts almost like a potassium electrode (this is known as the potassium effect).

<sup>28</sup> Briggs (8) and Söllner (118) state that such a mosaic would not admit both anions and cations as supposed by Höber. See also S. C. Brooks (21).

When we lead off from .01 M KCl to .01 M NaCl on the surface of the cell we obtain 85 mv. from which we calculate that the apparent mobility of  $K^+$  is about 40 times that of  $Na^+$  (94). All this disappears when the cells are washed for several days in distilled water (105). We then find that the water contains substances which can be extracted from it with petroleum-ether and which restore the potassium effect when redissolved in water and applied to the surface (40, 106). Hence it is evident that an organic substance is dissolved out by the distilled water which is responsible for the potassium effect. For convenience, this substance or group of substances may be called *R*. The nature of *R* is unknown but it has been found that the potassium effect can be restored by such substances as  $NH_3$  (101) and adrenalin (106); this does not seem to be primarily a question of alkalinity for such bases as aniline, toluidine, and alkaloids do not restore the potassium effect.

It would seem that we might imitate the non-aqueous protoplasmic surface by taking an indifferent substance and dissolving guaiacol in it. The guaiacol would enable it to distinguish electrically between potassium and sodium (though not to such an extent as the *Nitella* cell). In contact with distilled water the guaiacol would come out and leave the indifferent substance which would be unable to distinguish between them.

Cells of *Nitella* behave very much like nerve fibers in that they can be stimulated electrically to give action currents. The irritability disappears after exposure to distilled water (103); it thus acts like the potassium effect and can be restored in somewhat the same way so that it probably depends on the presence of a substance or a group of substances.

Another illustration is seen in the effect of guaiacol on *Valonia* which changes the order of apparent ionic mobilities in the surface from  $K > Cl > Na$  to  $Na > Cl > K$  (102).

#### CONCLUSION

The facts and principles here set forth are the results of investigations of large cells which offer special advantages for such studies. How far they are applicable to other cases remains to be seen.

This brief outline indicates progress in dealing with some very

interesting variables. It also gives a hint of the host of problems awaiting solution.

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## EXPLANATORY NOTES

BY THE EDITORS

*Adapted, in part, from Hackh's "Chemical Dictionary"*

acid: a chemical compound which yields hydrogen ions,  $H^+$ , when dissolved in water or a compound whose hydrogen can be replaced by metals or basic radicals or which reacts with bases to form salts and water.

acid, strong: an acid that ionizes greatly.

acid, weak: an acid that does not ionize greatly.

activity coefficient: a factor by which a concentration is multiplied to obtain a value called the activity which can be used in place of the actual concentration in equations set up for ideal solutions, i.e., solutions in which the ions or molecules of the solute have no more influence upon each other than they would have at infinite dilution (where the activity coefficient is unity). For very dilute solutions of univalent ions at  $25^\circ$  we have  $-\log \gamma = 0.506 \sqrt{C}$ , where  $\gamma$  is the activity coefficient and  $C$  is the molar concentration.

an: see ion.

base: a compound which yields hydroxyl ions,  $OH^-$ , in aqueous solution or  
 ba: a compound which reacts with an acid to form water and a salt.

cataphoresis: the motion of electrically charged particles suspended in a medium under the influence of an electric field.

chemical potential: the free energy per mole of a compound under constant conditions. The chemical potential of KOH in an aqueous solution is proportional to the  $[K][OH]$ , where the bracket denotes activity (*i.e.*, the concentration multiplied by the activity coefficient).

concentration: the strength of a solution. Molar concentration is the number of moles of a substance dissolved in one liter of solution and a mole is a gram-molecule, *i.e.*, a quantity of matter weighing  $M$  where  $M$  is the molecular weight.

concentration gradient: in a layer of solution with a depth  $x$  and a concentration  $C_1$  at the top and  $C_2$  at the bottom, the concentration gradient is  $\frac{C_1 - C_2}{x}$ . Strictly speaking, this applies only when the gradient is uniform.

dielectric constant: a dielectric is an insulator or non-conductor of electricity. The force exerted between any two charged point sources  $q_1$

and  $q_2$  is  $F = \frac{q_1 q_2}{D d^2}$ , where  $d$  is the distance separating them and  $D$  is the

dielectric constant (constant for any particular medium at constant temperature). Hence the attraction between  $Na^+$  and  $Cl^-$  is less in water ( $D=81$ ) than in guaiacol ( $D=14$ ) so that NaCl is a strong electrolyte in water and a weak electrolyte in guaiacol.

diffusion constant: the constant  $D$  in  $S = -Dqt \frac{C_1 - C_2}{x}$ , where  $S$  is the weight of substance diffusing in  $t$  seconds through a cylinder of length  $x$  and cross-section  $q$ , and  $\frac{C_1 - C_2}{x}$  is the concentration gradient, *i.e.*,

the difference between the concentrations at opposite ends of the cylinder divided by its length. Strictly speaking, this applies only when  $x$  is infinitely small; otherwise, it is only an approximation which becomes more exact as  $x$  becomes smaller.

Donnan equilibrium: a membrane equilibrium involving an unequal distribution of ions which are freely diffusible through a membrane when it has on one side ions which do not diffuse.

dissociation: the breaking apart of a molecule into ions by physical means.

dissociation constant: the product of the molar concentrations of the different participating ions divided by the molar concentration of undissociated molecules. For acids it is usually stated thus  $K = \frac{(H^+)(X^-)}{(HX)}$

where  $(H^+)$  and  $(X^-)$  represent the molar concentrations of the ions and  $HX$  represents the molar concentration of the undissociated portion ( $X$  represents any anion that may accompany the hydrogen ion).

electrolyte: any substance which dissociates into two or more ions, to a great or small extent, when dissolved in water or other solvent. Solutions of electrolytes thus conduct the electric current.

hydroxide: as here used signifies a compound yielding  $OH^-$  on dissociation: example, KOH.

ion: an electrically charged atom or more complex structure; cation if positively charged, as  $H^+$ ; anion if negatively charged, as  $Cl^-$ , since they travel in solution to the cathode or anode, respectively.

ionization: the breaking up of a molecule into two or more negatively and positively charged components or ions.

micron: one millionth of a meter.

mole: see concentration.

pH = the symbol for the logarithm of the reciprocal of the hydrogen ion activity (which becomes almost equal to the concentration in very

dilute solutions). Values indicate degrees of acidity or alkalinity: pH 7 is neutral, higher values more alkaline, lower values more acid.

partition (distribution) coefficient: the concentration of a substance in one phase divided by its concentration in another phase in equilibrium with it, *e.g.*,  $S_w/S_f$  may be the coefficient where  $S_w$  = the concentration of a substance in water and  $S_f$  = its concentration in chloroform when the three components are shaken together until equilibrium is reached.

$Q_{10}$ : a symbol whose value indicates how much the speed of a chemical reaction is multiplied for each  $10^\circ$  C. rise in temperature.

salts: substances resulting from reactions between acids and bases: example, sodium chloride.

temperature coefficient: any factor that indicates quantitatively the effect of temperature upon a property of matter or upon a process.

time curve of the first order: a curve expressing a reaction of the first order, *e.g.*, when a substance  $A$  decomposes to form  $B$ . If the initial amount of  $A$  be called  $a$  and the amount of  $B$  be called  $x$ , we have

$kt = \ln \frac{a}{a-x}$ , where  $t$  is time and  $k$  is the velocity constant of the reaction.

$a-x$  or  $x$  is plotted as ordinates and time as abscissae.

time curve of the second order: a curve expressing a reaction of the second order, *e.g.*, one in which two substances  $A$  and  $B$  unite to form  $C$ . If the amounts of  $A$  and  $B$  at the start are equal we may call this value  $a$  and the amount of  $C$  at any given time,  $t$ , may be called  $x$ . We then

have  $kt = \frac{x}{a(a-x)}$ , where  $k$  is the velocity constant of the reaction.

$a-x$  or  $x$  is plotted as ordinates and time as abscissae.

## SOME ASPECTS OF THE CYTO-GENETICS OF OENOTHERA

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*Oenothera* has long been known as one of Nature's most consistent non-conformists. In many features of its genetical and cytological behavior it has shown a wide departure from the rules which prevail in other organisms. It occupies, therefore, a position of major interest in the cyto-genetic field.

It was de Vries who first discovered that *Oenothera* is peculiar. His attention was called to the genus in 1886 when he found *Oe. lamarckiana* growing in great numbers in an abandoned field near Hilversum, in Holland. He found, among thousands of typical plants, occasional aberrant or exceptional individuals which seemed to have arisen as sports from the prevailing type. Collecting seed from typical plants, he grew many thousands of individuals in his experimental garden over a period of years and found the same aberrant types appearing from year to year in small percentages that had been present in the field, some of which bred true to their new characters. All of this seemed to de Vries to furnish a clue to the method by which new species are formed; and it was principally on the basis of his extensive study of *Oenothera* that he was led to formulate the celebrated Mutation Theory of Evolution (101) which assumed that species spring into existence suddenly, as a result of genetical modifications of major importance, rather than through gradual accumulation of many small variations.

But de Vries soon found that *Oenothera* is peculiar, not only because it produces sports, but also because it shows in certain aspects of its ordinary breeding behavior an anomalous condition, setting it apart from other organisms. de Vries was already becoming familiar, through his own experiments on a wide variety of material (98, 99, 100, 101), with the basic principles of heredity which he was later to find had already been set forth in Mendel's work. Thus, he was able to appreciate the uniqueness of *Oenothera* in certain aspects of its genetical behavior. Briefly, the anomaly which he found in *Oenothera* consists in the fact that a species which, when inbred, behaves like a pure species in that it breeds

perfectly true to type, may, nevertheless, behave like a hybrid when crossed with another species, for it may either produce progenies which include more than one kind of individual, as hybrids do, or it may produce different types of progeny when used as male and female parent (102, 103, 104, 105, 106). If such a plant is not hybrid, how can it give splitting progenies? On the other hand, if it is heterozygous or hybrid, how can it breed true? The anomaly seemed all the greater to de Vries when he learned that in many cases the individuals, resulting from such a cross between species, themselves breed true when selfed. According to Mendelian principles, as understood by de Vries, one would expect pure races, when crossed with each other, to produce uniform hybrid progenies, and one would expect the hybrids thus produced to give splitting progenies. But in the case of *Oenothera*, apparently pure species give splitting progenies and apparently hybrid individuals breed true. This can be made clear by a diagram contrasting the behavior of Mendel's peas with that in a selected case in *Oenothera*.

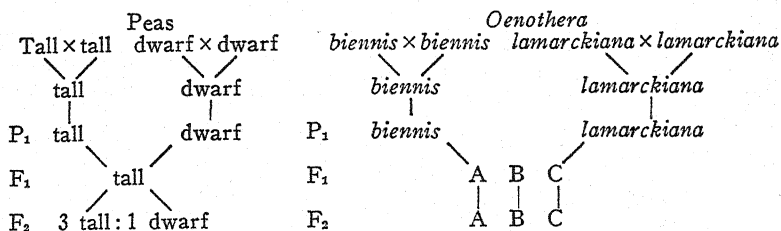


DIAGRAM 1

In peas, a pure tall strain, or a pure dwarf strain, remains true to type in an inbred line; and when these strains are crossed with each other, they produce a uniform F<sub>1</sub> because they themselves are homozygous. The hybrids thus produced, however, yield a splitting progeny in F<sub>2</sub>, if inbred. In *Oenothera*, the species *biennis* and *lamarckiana*, for instance, breed true when inbred, just as do the tall and dwarf peas. When crossed as above, however, they produce, not a uniform F<sub>1</sub>, but 3 distinct types of hybrid, and when these hybrids are selfed or inbred, each type, instead of yielding a splitting F<sub>2</sub>, breeds essentially true, except in flower size (76).

This was a situation which de Vries was unable fully and correctly to explain, his failure being due, in part, to the fact that he

was long unwilling to consider *Oe. lamarckiana* in any other light than that of a pure species, realizing what effect admission of the heterozygosity of *lamarckiana* would probably have upon the interpretation of the nature of its mutants, and hence upon the whole mutation theory.

The complete analysis and explanation, from the genetical point of view, of this anomalous behavior, was the work of other investigators. Bartlett (1), Cobb and Cobb and Bartlett (5, 33, 34) in America, and Renner (59, 60, 74-82) in Germany, arrived independently at the concept of complex-heterozygosity, which was to form the starting point for our present adequate understanding of the peculiarities of *Oenothera* breeding behavior. Building upon this concept as a foundation, Renner and his followers, in an extensive and masterly series of studies, thereupon proceeded to establish the correctness of this concept and to elucidate further peculiarities as well. It is largely the work, therefore, of Renner and his followers (Oehlkers 69-73, Rudloff 84-86, Gerhard 54) which has given us the clear and complete picture which we now have of the genetical situation in *Oenothera*.

This situation will now be summarized, as follows:

(1) All *oenotheras*, so far as known, have 14 chromosomes. According to the ordinary rules of cytological and genetical behavior, we should expect to find them behaving as though they had 7 independent pairs of chromosomes in meiosis, and corresponding to these, 7 independent linkage groups. However, we find that a great majority of the spontaneous races of *Oenothera* which have been studied genetically behave as though they had but one pair of chromosomes and one linkage group.

A plant which had but one pair of chromosomes would receive one chromosome from each parent. The entire paternal set of genes would be in one chromosome and the entire maternal set in the other. In reduction division, therefore, the paternal genes would separate from the maternal, and one-half of the germ cells would be found to contain the entire paternal inheritance, the other half would contain the entire maternal inheritance (except for crossing-over). If the plant were highly heterozygous (and all species of *Oenothera* which behave in this fashion are highly heterozygous), the two sets of genes and the two types of germ cell

would differ materially. Furthermore, except for crossing-over, one would expect to find the genes in each chromosome sticking together generation after generation—there would be no more than 2 sets of genes in the germ cells of a given race, crossing-over excepted.

Now this is just the way in which most species of *Oenothera* behave. In these species, all (in some cases, not quite all) of the genes are united into a single linkage group—at least this is true of those genes which are not necessarily common to all species of *Oenothera*. All of the paternal genes of this group are separated in reduction division from all of the maternal genes, and there are produced but 2 kinds of germ cells, those containing the entire paternal set, and those containing the entire maternal set. These sets or "complexes," as Renner has called them, are passed on intact from generation to generation, and hence maintain their identity indefinitely. They are thus in a sense as much entities as are the species themselves; and hence Renner has given them names, such as *velans* and *gaudens*, making up *lamarckiana*, or *rigens* and *curvans*, making up *muricata*. Each species is a heterozygote, composed of 2 complexes; or in the terminology of Renner, it is a complex-heterozygote. But here is where the anomaly is found—*Oenothera*, while it acts in most cases as though it had but one pair of chromosomes (since it has but one linkage group), has in reality 14 chromosomes.

(2) All species in which the genes are mostly or entirely confined to a single linkage group, are highly heterozygous. In spite of this, however, they breed true, as de Vries originally observed. This is due to the presence of balanced lethals, which may be of 2 sorts: (a) the first sort prevent the development or functioning of those eggs or sperms, as the case may be, which carry a given set or complex of genes. These are sometimes called gamete lethals, although they probably function, not in the gametes proper, but in the gametophyte generation which precedes gamete formation or even in the spores which produce the gametophytes. They may do nothing more, in some cases, than inhibit pollen tube growth or prevent the microspores in which they are found from competing successfully in embryo sac development. (b) The second sort, the so-called zygote lethals, prevent the development of individuals which have received the same complex from both



parents. The result of either kind of lethal is to make only one combination of complexes possible in any given generation of an inbred line, namely, that which duplicates the previous generation; and hence the race, although heterozygous, is forced to breed true. (see diagram 2).

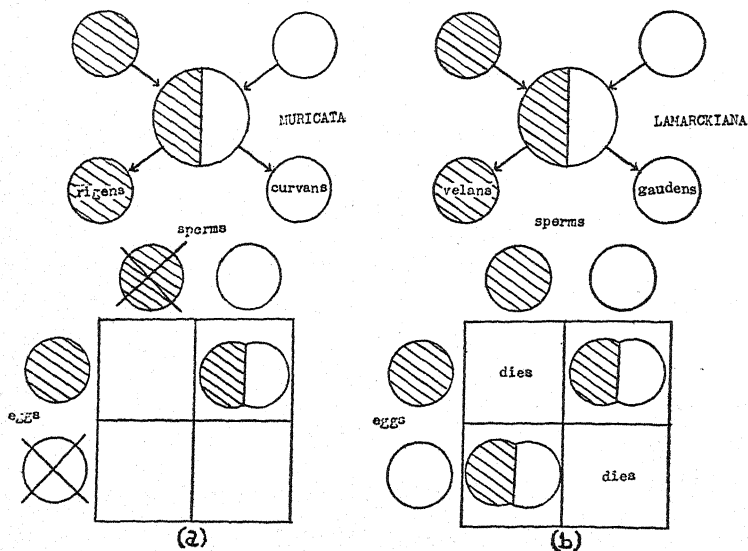


DIAGRAM 2

- (a) "Gamete lethals" in *muricata*. *Rigens* is prevented by a lethal from functioning as sperm; *curvans* is prevented from functioning as egg. Hence only one combination is possible in each generation, *rigens* · *curvans*.
- (b) Zygote lethals in *lamarckiana*. Both *velans* and *gaudens* function both as sperm and egg; but *velans* · *velans* and *gaudens* · *gaudens* die because these complexes possess each a zygote lethal. Hence only *velans* · *gaudens* survives.

(3) Although complex-heterozygotes breed true when selfed, and thus act as though they were pure, they show their heterozygosity when crossed with other species which have other lethals. Those species which, like *lamarckiana* or *grandiflora*, produce 2 kinds of functional sperms and 2 kinds of functional eggs, tend to produce twin or multiple types in their progenies; those which, like *muricata* or *chicaginensis*, produce but one kind of functional sperm and one kind of functional egg, produce unlike reciprocals. Some examples will make this clear (see diagram 3).

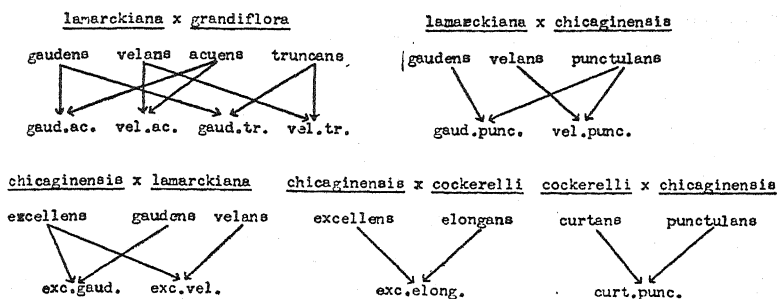


Diagram 3

(a) If one crosses *lamarckiana* with *grandiflora*, both parents having 2 types of sperms and 2 types of eggs, there will be 4 classes of progeny, thus showing the heterozygous character of both parents. (b) If *lamarckiana* is crossed with *chicaginensis*, only 2 classes of progeny will be formed, whichever way the cross is made, since *chicaginensis* has but one kind of functional sperm and egg. Since, however, its egg complex is very different genetically from its pollen complex, the 2 classes produced when *chicaginensis* is female parent will be very different from the 2 classes produced when it is used as male parent. Thus, the heterozygosity of *chicaginensis* is made clear by the production of unlike reciprocals, whereas the heterozygosity of *lamarckiana* is shown by the production of twins in both reciprocals.

(c) If *chicaginensis* is crossed with *cockerelli*, only one class of progeny will be found, whichever way the cross is made. The only clue to the heterozygosity of the parents is the fact that the reciprocal hybrids are unlike. These crosses alone, however, are not enough to tell whether both, or only one, of the parents is heterozygous.

In all these cases, one curious fact is to be noted, namely, that individuals receive inheritance from only 2 of their grandparents, and not from all 4, as is usual among other organisms. In the first example given, each individual has received, through each parent, either the entire inheritance which the latter has received from its mother, or the entire inheritance which it has received from its father; it has inherited from one paternal and one maternal grandparent only. In the other cases, individuals whose mother

is *chicaginesis* or *cockerelli*, for instance, can inherit, on the female side, only from the maternal grandmother. They cannot possibly receive inheritance from the maternal grandfather, except through crossing-over. On the other hand, if either *chicaginesis* or *cockerelli* is male parent, their progeny can inherit on the male side only from their paternal grandfather, and not from their paternal grandmother. This is a curious result of the linkage of all heterozygous genes into one group, coupled with the presence of balanced lethals.

Other examples might be cited to cover certain species which occupy an intermediate position between the *lamarckiana* type, with 2 kinds of functional sperm and egg, and the *chicaginesis* type, with but one kind of each. There are species, like *biennis* or *suaveolens*, in which both complexes can function as egg (one often but rarely), but in which only one complex is able to function as sperm. On the other hand, races are known in which the reverse is true; only one complex functions in the egg, but both may be transmitted through the pollen (the egg complex much less frequently than the other, however). Whatever the condition with respect to the appearance or non-appearance of one or both complexes in sperm and egg, however, the principle is illustrated by all that the various complex-heterozygotes, although they breed true when selfed, show their heterozygosity when outcrossed by the production of twin or multiple types, or of unlike reciprocals.

(4) The presence of lethals naturally leads to a considerable percentage of sterility. Forms with gamete lethals usually show considerable pollen sterility, inasmuch as one of the complexes is unable to function on the male side. Such forms, however, show little or no seed sterility when selfed, since all the functional eggs are capable of fertilization by any of the sperms, and consequently all zygotes are theoretically viable. On the other hand, complex-heterozygotes with zygote lethals show at least 50% seed sterility, since half the zygotes produced by inbreeding are homozygotes, and therefore inviable.

(5) We have seen so far that most species of *Oenothera* are highly heterozygous and are characterized by the union of all or nearly all of the genes, not common to all species, into a single linkage group, which group contains a balanced lethal system that prevents the production of homozygotes and thus enforces a condi-

tion of permanent heterozygosity upon these races. In view of this union of all or most of the genes in the various species, it is surprising, therefore, to discover that in the case of many *hybrids* between species, certain genes, which were linked together in the parents, are no longer linked in the hybrids—in other words, *the single linkage group in the parents has been broken up into two or more smaller ones in the hybrids*. In illustration, we may use certain data from the work of Renner (81) which are presented in diagram 4. At the top is listed a number of genes, and to the

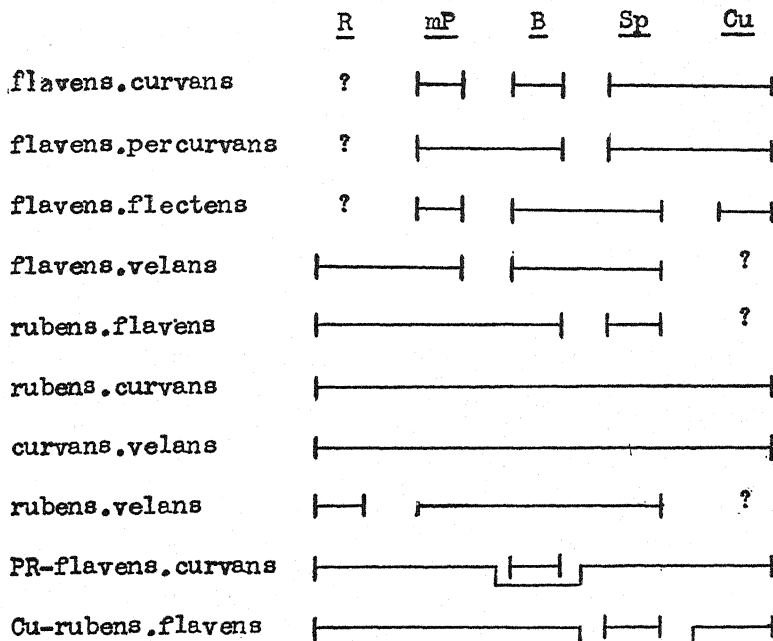


DIAGRAM 4

left are various hybrid complex-combinations, obtained by crossing certain species. In *flavens · curvans* at least 3 linkage groups are present, with *mP* in one, *B* in another and *Sp* and *Cu* in the third. In *flavens · percurvans*, however, *mP* and *B* are in the same linkage group. In *flavens · flectens*, *Cu* is independent of *Sp*, the latter being linked with *B*. In *PR-flavens · curvans*, *B* is independent of the rest of the genes, which are all linked together. Similarly, as will be seen, the rest of the hybrids have each their own charac-

teristic number of linkage groups and distribution of these genes throughout the groups.

Now this is an utterly anomalous situation which cannot be understood on the basis of the conventional explanation of linkage, for in ordinary linkage, genes which are in the same linkage group at one time will always remain in this group, unless some accident, such as translocation or fragmentation, occurs, to alter the group. The alteration of linkage relations which occurs in *Oenothera*, however, is not in the nature of an accident, for it always occurs in a given way after a given cross, no matter how often the cross may be repeated, each hybrid having always its own characteristic number of linkage groups and distribution of genes throughout these groups.

The chief points have now been summarized at which *Oenothera* fails to abide by the ordinary rules of hereditary behavior. To recapitulate briefly: (1) although they have 14 chromosomes and should, therefore, have, theoretically, 7 independent linkage groups, most *oenotheras* behave as though they had but a single group. With respect to this one group, they are highly heterozygous and thus produce two and only two kinds of gamete from the standpoint of the gene complexes which are carried. (2) By means of balanced lethals, they manage to breed true when selfed; nevertheless, (3) the fact that they are highly heterozygous is shown in outcrosses. (4) The presence of balanced lethals results in a high degree of gametic or zygotic sterility. (5) When species are crossed, genes which were linked in the parent species often become independent in the hybrids between these species; there are in such hybrids two or more linkage groups in place of the original single one, and the number of linkage groups in a given hybrid is constant.

With this all-too-brief outline of the principal genetical peculiarities of *Oenothera*, we may next consider the question as to the physical basis of these phenomena. If the chromosome theory of inheritance is correct, it ought to be possible to find an adequate explanation of these peculiarities in terms of chromosome behavior.

Not all of the phenomena which have been mentioned above suggest unusual chromosome behavior. However, two of *Oenothera's* peculiarities are such as to raise doubts, to say the least, as to the normality of chromosome behavior in the genus. These are (1)

the linkage of all or most of the genes into a single linkage group, and (2) the alteration of linkage relations which takes place in species-hybrids. How are these to be explained?

The first of these phenomena may be explained in one of two ways. It is possible to assume, as one alternative, that all or most of the genes governing visible and contrasting characters in *Oenothera* reside in a single chromosome pair, that the other 6 pairs are almost or entirely empty of genes, or at the most carry only such genes as are common to all known gene complexes (91-95). According to this explanation, genetical linkage in *Oenothera* is no different from linkage in *Drosophila*—genes are linked because they reside in the same chromosome, and for no other reason.

This sounds like a plausible hypothesis but there are two objections to it. First, it is hard to understand how practically all heterozygous genes, and genes for visible differences between individuals and races, should come to lie within a single chromosome, and the same chromosome, in all these species. Secondly, this hypothesis is unable to account for the breaking up of the single linkage group which occurs in species-hybrids. It is obvious that genes which lie within a single chromosome must remain linked permanently, or until the chromosome becomes altered through fragmentation or translocation. They cannot reside in one chromosome in one generation and be scattered among a number of chromosomes in the next generation. Inclusion within a single chromosome cannot, therefore, be the basis for a type of linkage that varies from generation to generation.

The second alternative must, therefore, be considered, which is to the effect that the genes, apparently linked in *Oenothera* into a single large group, are actually scattered throughout the various chromosomes, but that there is some mechanism which ensures that all chromosomes of paternal origin will pass to one pole in the reduction division, and all those of maternal origin will pass to the other pole, thus resulting in the presence of the entire paternal gene complex in one-half the germ cells, and the entire maternal complex in the other half. This explanation seems at first sight even more bizarre than the other, but let us examine into chromosome behavior in the genus to see whether there is any ground for assuming that such a situation prevails.

It has been known since 1908 that the chromosomes of many *Oenothera*s do not behave normally in meiosis. Gates (50-52) and Davis (39-41) showed that they have a tendency to remain in an unpaired condition, and to separate to the poles without having shown any marked association. It was not until later, however, (13-18, 42, 55) that it was realized that chromosome behavior in *Oenothera*, while often different from that found in other organisms, is, nevertheless, regular and consistent, following certain definite rules with great constancy. The situation, as it is now known to exist in those races which exhibit peculiarity, is quickly explained. In a large majority of the species which have been studied cytologically, most or all of the chromosomes, instead of pairing in late prophase of the first meiotic division, are found attached end to end to form closed circles, each circle having an even number of chromosomes (fig. 1). The number of chromosomes in a circle,

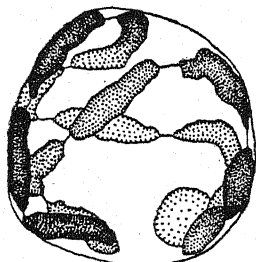


FIG. 1



FIG. 2

and the number of circles, is always constant in a given race. When metaphase sets in, the circles remain intact and are gathered as a unit into the center of the spindle. Adjacent chromosomes then begin to move in opposite directions—toward opposite poles (fig. 2). For a time after this movement begins, they hang suspended between the poles, the force toward the poles being apparently insufficient to cause the attached chromosomes to separate from one another. Then breakage occurs and the chromosomes are quickly separated. As a result, adjacent chromosomes pass, as a rule, to opposite poles. There are, of course, exceptions to this rule—accidents happen in a percentage of cases which alter the normal procedure. On the whole, however, adjacent chromosomes are separated to opposite poles in a very large majority of the cells.

This completes a brief outline of some of the genetical idiosyncracies of the *oenotheras* on the one hand and their cytological peculiarities on the other. The question now arises as to whether there is any causal relation between them. Can the linkage of all or most of the genes into a single linkage group be explained by the linkage of the chromosomes into chains? Can the alterations in linkage, which occur when crosses are made, be explained in terms of chromosome behavior?

It was early realized (14, 16, 18, 20, 21, 55, 72) that chromosome linkage could, indeed, explain the genetical peculiarities of the genus, provided one were to make a single assumption, namely, that chromosomes of paternal and maternal origin occupy alternate positions in the circle (diagram 5). It is easy to see that if they



DIAGRAM 5

Chromosomes of paternal origin (white) alternate with chromosomes of maternal origin (black) in the chain, and consequently are separated to opposite poles in reduction anaphase.

do occupy alternate positions, the separation of adjacent chromosomes to opposite poles, which normally occurs, would necessarily send all the paternal chromosomes to one pole, and all the maternal ones to the other; and thus one-half of the germ cells would have the complete paternal gene complex and one-half would receive the complete maternal set, which is what actually occurs in *Oenothera*.

This alternation of paternal and maternal chromosomes was, of course, a pure assumption at first; but fortunately, it was one that was capable of being tested. It was by this time becoming known that hybrids between species may have many sorts of arrangement of their chromosomes into circles and pairs (19, 23, 73). There are, as a matter of fact, 15 different arrangements possible in diploids with 14 chromosomes (see chart 1) all of which have now been found at least once among the many hybrids which have been studied. In some of these arrangements, the chromosomes are all or mostly in circles, in others they are mostly paired. Now if the extensive linkage of all or most of the genes in *Oenothera* is dependent upon the end-to-end union of the chromosomes in which the linked



genes reside, it is obvious that such linkage can last only so long as the chromosomes are thus united. Consequently, if we should cross two species in which the chromosomes, and hence the genes, were all united, and should find that the chromosomes were no longer united in the hybrid, but were mostly or entirely in a paired condition, then we should expect on the basis of this assumption to find the genes in the now separated chromosome pairs no longer linked—we should expect them to show independent assortment, and hence should look forward to a varied progeny if these hybrids were selfed. On the other hand, if the chromosomes in the hybrids were found to be united as they are in the parental races, we should expect to find the genes also still linked, which would be evidenced by the fact that such hybrids would breed true in following generations—would behave, in other words, just like the parental races. Obviously, then, the way to find out whether chromosome linkage results in extensive genetical linkage or not would be to cross species, determine the arrangement of chromosomes in the hybrids, and then grow the progenies of the hybrids to see whether a correlation exists between the degree to which the chromosomes are linked or free in the hybrids, and the degree to which the genes are linked or free.

The first study of this kind was made by Oehlkers (73) who showed that when *suaveolens* is crossed by *strigosa* (both species being true-breeding complex-heterozygotes) twin hybrids are produced, of which '*albicans* · *stringens*, with  $\odot^1$  12, breeds true, except for the independent splitting of one factor, whereas *flavens* · *stringens*, with  $\odot$  4 and 5 pairs, shows independent segregation in respect to at least 3 factors. This study was followed later by an extensive series of tests, performed by Cleland and Oehlkers (31, 32) and Renner and Cleland (83). In these tests, some 50 different species-hybrids were grown, their chromosome configurations determined and their breeding behavior analyzed. This is not the place to review these studies in detail—it will suffice to emphasize the fact that the results were entirely confirmatory of the hypothesis that the linking together of the chromosomes, with paternal and maternal chromosomes alternating, and the separation of adjacent chromosomes to opposite poles, constitute the physical

<sup>1</sup> This symbol denotes a ring composed of the indicated number of chromosomes.

basis for the extensive genetical linkage found in *Oenothera*. Hybrids with a circle of 14 or a circle of 12 chromosomes were found to breed essentially or entirely true; at the other end of the scale, hybrids with 4 or 5 pairs gave progenies which showed much segregation, and those which occupied an intermediate position in the scale of chromosome configuration gave an intermediate amount of splitting in  $F_2$ . A close correlation was thus found between the number of chromosome groups present in a hybrid and the amount of splitting shown by the progeny of this hybrid. From these and subsequent studies (24, 30, 44, 47, 48, 49, 82, 86), it seems clear that the number of linkage groups in a plant depends, not upon the number of chromosomes or chromosome pairs, but upon the number of chromosome groups, whether these be circles or pairs. Thus, a plant with  $\odot$  14 has but one linkage group, one with  $\odot$  10 has one large and 2 small groups, etc. *Circles, then, form the basis of single linkage groups, just as pairs do, and the reason why a circle forms the basis of only one linkage group is that the paternal and maternal chromosomes in the circle alternate, and adjacent chromosomes go to opposite poles.*

In line with this evidence, is the evidence from the spontaneous races themselves. Some 54 distinct species or races have been studied cytologically up to the present. Of these, 22 have been proved to be complex-heterozygotes, whose genes are all, or nearly all, linked into a single group; and 19 are known to be races whose genomes are essentially similar, which carry no lethals, and which show independent segregation of their heterozygous genes. It is a striking fact that all 22 of the complex-heterozygotes have large circles, whereas all of those which are not complex-heterozygotes have mostly or entirely paired chromosomes. There is, therefore, in species as well as in hybrids, clear-cut evidence to show that extensive linkage is present only when the chromosomes are mostly or entirely linked, and absent when chromosome catenation is absent. The perfect correlation thus observed between genetical and cytological behavior cannot be purely a coincidence, for the number of forms which show it is much too great to allow of such an interpretation.

A further, and if anything, a more convincing proof of the causal connection between chromosome concatenation and extensive genetical linkage was furnished when it became known that the

union of chromosomes end-to-end in meiosis is a result of the phenomenon of segmental interchange. This realization, as we shall show, made it absolutely necessary to adopt the conclusion that paternal and maternal chromosomes alternate in the circle, and hence that the paternal and maternal gene complexes are segregated intact from each other in meiosis. It will be advisable at this point, therefore, to indicate briefly the nature of the proof that segmental interchange has been responsible for circle formation, and then to show that acceptance of segmental interchange as a fact makes it absolutely necessary to accept also the alternation of paternal and maternal chromosomes in the circle, and hence the causal relation between chromosome linkage and extensive genetical linkage.

The concept of segmental interchange (or reciprocal translocation) originated with Belling (2, 3) who adopted it to explain the presence of a circle of 4 chromosomes in a hybrid between two lines of *Datura stramonium*. The concept briefly is this: homologous chromosomes attract each other in synapsis, through some affinity which exists between homologous regions or, more specifically, between homologous genes. Now if a portion of one chromosome exchanges with a portion of another non-homologous chromosome, the pairing of homologous regions in synapsis will then result in the formation of a circle of 4. This will be made clear by a diagram in which homologous end-segments are indicated by corresponding numbers (diagram 6). Further interchanges between chromosomes in the circle and paired chromosomes will increase the size of the circle.

Belling (2) suggested that this process might explain circle formation in other genera, including *Oenothera*; and Håkansson (56, 57) and Darlington (35, 37, 38), taking up this suggestion, applied it more specifically to *Oenothera*, and showed that it was capable of explaining the situation in this genus. Meanwhile, Emerson and Sturtevant (46, 48, 97), as well as Blakeslee and the writer (4, 28, 29), were engaged independently in an effort to test the correctness of this hypothesis as applied to *Oenothera*. These authors realized that, if segmental interchange has occurred, each complex should have its own specific arrangement of end segments throughout the chromosomes, and thus, knowing the arrangements in certain complexes, it should be possible to predict the chromosome configura-

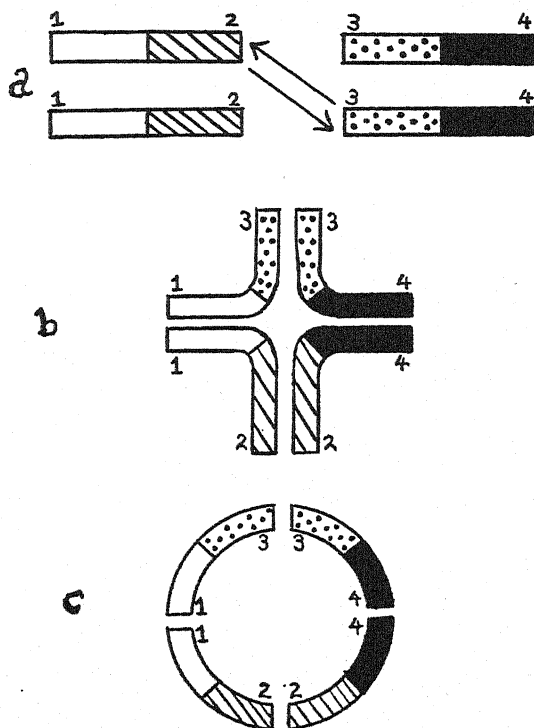


DIAGRAM 6

- (a) Interchange between non-homologous chromosomes.  
 (b) Synapsis between interchanged and normal chromosomes.  
 (c) Resultant circle-formation.

tions which would be found when these complexes are combined in the formation of hybrids. They set out, therefore, to make and test such predictions, in an effort to test the correctness of the segmental interchange hypothesis.

To show how these predictions can be made, a single example will be presented. Suppose we take the case of *rigens* · <sup>h</sup>*Johansen* (*rigens* is the egg complex of *muricata*, <sup>h</sup>*Johansen* is the single type of genom produced by the homozygous California race, known at present as "*Johansen*"). The configuration of this hybrid was successfully predicted some years ago (25). The argument employed at that time was long and involved, owing to the fact that it was not then known that *acuens* gives ⊙ 4 and 5 pairs with *flavens* (27). Knowing this fact, a much simpler line of reasoning

is sufficient to show that *rigens* · <sup>h</sup>*Johansen* must have  $\odot$  8 and 3 pairs; and since this simpler reasoning will illustrate the method as well as the more complex reasoning originally used, it will be employed.

We start with a knowledge of the segmental arrangements of certain complexes as a background; formulae for these complexes are as follows (each chromosome is represented by 2 digits joined by a dot, each digit representing an end segment):

|                                 |       |       |     |     |     |      |       |       |
|---------------------------------|-------|-------|-----|-----|-----|------|-------|-------|
| <sup>h</sup> <i>hookeri</i> has | ..... | 1·2   | 3·4 | 5·6 | 7·8 | 9·10 | 11·12 | 13·14 |
| <i>flavens</i>                  | ..... | 1·4   | 3·2 | 5·6 | 7·8 | 9·10 | 11·12 | 13·14 |
| <i>velans</i>                   | "     | ..... | 1·2 | 3·4 | 5·8 | 7·6  | 9·10  | 11·12 |
| <sup>h</sup> <i>Johansen</i>    | "     | ..... | 1·2 | 3·4 | 5·6 | 7·10 | 9·8   | 11·12 |
| <i>acuens</i>                   | "     | ..... | 1·4 | 3·2 | 5·6 | 7·10 | 9·8   | 11·12 |

*Rigens* gives  $\odot$  6 and 4 pairs with <sup>h</sup>*hookeri*;  $\odot$  4,  $\odot$  6, 2 pairs with *flavens*;  $\odot$  8, 3 pairs with *velans*; and  $\odot$  4,  $\odot$  8 and 1 pair with *acuens*. Its configuration with <sup>h</sup>*Johansen* is the subject of prediction.

Since *rigens* gives 4 pairs with <sup>h</sup>*hookeri* and only 2 pairs with *flavens*, it has 2 more chromosomes with ends arranged as in <sup>h</sup>*hookeri* than it has chromosomes whose ends are arranged as in *flavens*. But there are only 2 <sup>h</sup>*hookeri* chromosomes which are not like those in *flavens*; consequently, these must be the ones which <sup>h</sup>*hookeri* has in common with *rigens*, but which *flavens* does not have. *Rigens*, therefore, has 1·2 3·4. The fact that it has 1·2 3·4 will account for the presence of  $\odot$  4 in *rigens* · *acuens*. But *rigens* gives with *acuens* not only  $\odot$  4, it gives also  $\odot$  8, and the latter must involve 4 of the last 5 chromosomes, because the first two are involved in the  $\odot$  4. Since the last 5 chromosomes, however, are the same in arrangement in <sup>h</sup>*Johansen* and *acuens*, any configuration which *rigens* gives with the last 5 chromosomes of *acuens*, it will also give with the last 5 chromosomes of <sup>h</sup>*Johansen*—in other words, it will give  $\odot$  8 with <sup>h</sup>*Johansen* also. But both complexes having 1·2 3·4, the entire configuration in *rigens* · <sup>h</sup>*Johansen* must be  $\odot$  8 and 3 pairs.

Several months after this prediction was arrived at, opportunity came for examining this hybrid, and it was found to have the predicted configuration. More than 40 such predictions have been made, tested and published by Emerson and Sturtevant (46, 48, 49, 97), Cleland and Blakeslee (4, 28, 29) and the writer alone

(24, 25), and the only one of these that was wrong was one which was based upon a mistake in data. When this mistake was detected it was found that the revised prediction which alteration in the data made necessary was correct (27). We may say, therefore, that there have been no failures in predicting the chromosome configurations of various hybrid combinations; there has been, on the other hand, 100% success.

This can mean but one thing, namely, that the premises upon which the arguments have been based which have resulted in these predictions are correct. The premises are: (1) that the gene complexes making up the various species have diverse arrangements of the end segments of their chromosomes; (2) that each complex has its own specific and characteristic arrangement which it retains indefinitely or until a successful segmental interchange alters it. But naturally the existence of so many different arrangements of ends can have come about in only one way, namely, through a process of segmental interchange. The only other alternative is to give up the evolutionary point of view entirely in accounting for the origin of the various *Oenothera*s and their complexes. We may say, therefore, that segmental interchange has been shown to be not only a possible explanation for circle formation in *Oenothera*, but the *only* possible explanation compatible with an evolutionary point of view.

Let us now return for a moment to the question as to whether the union of chromosomes end to end is responsible for the extensive linkage of genes so characteristic of this genus. According to the segmental interchange concept, the force which holds the chromosomes together in a circle is the same as that which holds paired chromosomes together, namely, the force exhibited during synapsis, whatever that force may be. But chromosomes which synapse even in part are always, in a diploid, the one of paternal, the other of maternal origin. Since, therefore, the force which holds together adjacent chromosomes in a circle is synapsis, it must necessarily follow that these are alternately of paternal and maternal origin. This necessary and unavoidable conclusion places our former assumption upon an unassailable foundation. Each chromosome occupies a definitive position within the circle, next to the two chromosomes which carry end-segments homologous with its own. If a given chromosome is paternal in derivation,

the two with segments homologous with its own must be maternal, and *vice versa*. Paternal and maternal chromosomes, therefore, alternate and the separation of adjacent chromosomes to opposite poles, which is an observed fact, must necessarily result in the separation of all paternal chromosomes and genes in the circle to one pole, and all maternal chromosomes and genes to the other. This accounts for complex-formation and for the apparent linkage of all genes in these chromosomes into a single linkage group as long as the chromosomes remain attached. It may be asserted, therefore, that the connection between chromosome concatenation and extensive genetical linkage in *Oenothera* is completely established.

#### PHYLOGENETIC IMPLICATIONS OF CYTO-GENETIC FINDINGS

A large number of species and hybrids of *Oenothera* have now been studied cytologically. From the standpoint of phylogeny, it is valuable, first of all to inquire into the frequency with which the various configurations have appeared during these studies.

*Species.* A total of 54 distinct races belonging to the sub-genus *Onagra* (which includes all the forms so far studied genetically) have been examined cytologically. These races fall into two very distinct groups from the standpoint of chromosome configuration: 35 of them show large circles (29 have  $\odot$  14, 4 have  $\odot$  12 and 2 have  $\odot$  6,  $\odot$  8); and 19 show small circles or none (10 have 7 pairs, 9 gave a variety of configurations in the first garden-grown generation, most individuals having 7 pairs or  $\odot$  4, a few 2  $\odot$ s 4 or  $\odot$  6). Seven of the 15 possible chromosome configurations have been conspicuous by their absence in natural races. This segregation into 2 clearly distinct groups (one with large circles and one with small circles or none) is extremely suggestive and will be referred to again later.

*Hybrids.* The chromosome configurations of 282 different hybrid combinations have been determined, this figure including only those hybrids whose parents have both been natural races, as opposed to mutants in pedigreed lines. Every one of the 15 chromosome configurations possible in diploids has been obtained within this assemblage, as will be seen from the solid line curve in chart 1. The hybrids so far studied, therefore, run the entire gamut from those with no pairs to those with nothing but pairs;

a fair majority of forms having fewer than 3 pairs, but a goodly proportion having from 3 to 7 pairs.

What, if any, phylogenetic significance can be found in these facts? Obviously, the presence of a large circle in a plant means that the sets of chromosomes uniting to produce this plant are quite dissimilar from the standpoint of segmental arrangement; on the other hand, the presence of mostly or entirely paired chromosomes is indication of the presence in the plant of chromosome sets whose segmental arrangements are similar or identical. We may now ask ourselves whether similarity in segmental arrangement is significant from the standpoint of phylogeny. Similarity in external morphological characters suggests, in many cases, phylogenetic affinity; does similarity in segmental arrangement have a similar significance?

Similarity in segmental arrangement may or may not have phylogenetic significance, depending upon whether segmental interchanges can occur between any two ends with more or less equal facility or whether they are restricted. If they are to some extent restricted, if some exchanges can occur much more easily than others, if many exchanges are perhaps impossible, it is to be expected that similarities in segmental arrangement will often be found among the complexes present in nature and that, therefore, little or no phylogenetic importance can be attached to such similarity. To take an extreme example: suppose that interchanges can occur only between chromosomes 1-2 and 3-4; then we can have, in addition to the original segmental arrangement (1-2 3-4 5-6 7-8 9-10 11-12 13-14), only 1-4 3-2 and (or) 1-3 4-2, the other chromosomes being alike in all complexes. Any cross we might make, in this event, no matter what the species used in the cross, would yield mostly paired chromosomes and would bring together genomes with similar segmental arrangements. This, of course, is an extreme example, but it serves to bring out the point that narrow restriction in the number of possible interchanges must result in reduction in the number of possible segmental arrangements, and hence must increase the chances of paired chromosomes being found when the various complexes are brought into combination. Similarity in segmental arrangement would not in this event necessarily mean close phylogenetic affinity.

On the other hand, it may be that interchanges can occur at ran-





are relatively great (in contrast to 1 chance for the production of 7 pairs, there are 13,440 chances for  $\odot$  12, 4,032 chances for  $\odot$  10); on the other hand, the chances for production of many pairs are small (but 21 chances for 5 pairs and 140 chances for 4 pairs in comparison with 23,040 chances for  $\odot$  14). Obviously, if interchanges occur with any approach to randomness in nature, the chances of finding complexes with similar segmental arrangements (complexes which will give many pairs with each other) will be relatively slight.

This being the case, similarity in segmental arrangement, unless found with extreme rarity, will have to be, in general, ascribed to phylogenetic affinity. Complexes are similar in segmental arrangement because they have had a relatively recent common origin from which they have not as yet had time to deviate materially.

Now, similarities in segmental arrangement are not found with extreme rarity but have been observed rather frequently. Does this mean that interchanges are restricted as to the ends between which they can occur, or is it true that interchanges can occur between any two ends, and that similarities in arrangement are due to a close relationship between the complexes which show them?

All of the evidence so far accumulated seems to indicate that complexes with similar segmental arrangements are indeed closely related in most cases and that, therefore, similarity in segmental arrangement is on the whole indicative of close relationship. This evidence will now be briefly reviewed.

(1) *Oenothera* races which are highly heterozygous have large circles, whereas those which are mostly or entirely homozygous have mostly or entirely paired chromosomes. Thus, all of the 35 races with large circles which have been analyzed sufficiently for a statement to be made, have been found to be complex-heterozygotes, their genomes being dissimilar; on the other hand, the 19 races with small circles or none have turned out to have genomes which are identical, or at least similar to each other. Races which are intermediate in chromosome configuration have been conspicuous by their absence. This all seems to suggest that the presence of mostly paired chromosomes in a wild race is an indication of close relationship between the genomes making up this species.

(2) Occasionally, one of the complexes of *lamarckiana* ex-

changes a portion of itself for a corresponding portion of the opposing complex, giving up its lethal but receiving no lethal in return from the other complex. The modified alethal complex thus formed is then capable of existence in double dose. Consequently, a mutant arises in which this complex is present twice. Naturally, such a mutant is completely homozygous. It is interesting to note that all such mutants (there are 4 known at present) have 7 pairs of chromosomes. Then there are species in which one complex is alethal or easily rendered alethal, e.g., *acuens* of *grandiflora* or *flavens* of *suaveolens*. Individuals are occasionally produced by these species and survive, in which these complexes are present twice. Such individuals are again naturally quite homozygous, except perhaps for the lethal, and they also have wholly paired chromosomes. In all these cases, the presence of wholly paired chromosomes, i.e., complete identity in segmental arrangement, goes along with practical or absolute identity in genetic composition.

(3) A third line of evidence is derived from a comparative study of complexes in different species. Renner has for many years been studying various complexes genetically, analyzing them from the standpoint of their gene content. He has found certain cases in which complexes, which are in different species, are, nevertheless, more closely related to each other than they are to the complexes which are their normal associates (59, 80).

In the meantime, the writer has been studying many of these same complexes from the standpoint of the chromosome configurations which they give when brought into combination with each other, and has found that the complexes which Renner considers to be closely related give mostly pairs when united and, therefore, have similar segmental arrangements, whereas the complexes which he regards as quite unrelated give large circles with each other (23). Here again is clear-cut evidence that the presence of numerous pairs in a complex-combination is evidence of close relationship between the complexes involved.

All of these lines of evidence, therefore, seem to indicate that the number of pairs present in a form is a rough indication of the degree of relationship existing between the complexes making up this form, which means that chromosome configuration becomes an important tool for the study of *Oenothera* phylogeny, affording, as

it seems to do, a simple and rapid method of determining close relationships between complexes. Such a tool should be a very useful supplement to taxonomic and other approaches to the problems of species relationship.

With a view to testing the usefulness of this tool, therefore, and utilizing it, so far as possible, in the solution of problems of *Oenothera* phylogeny, Prof. P. A. Munz of Pomona College and the writer have begun a systematic survey of the *oenotheras* belonging to the sub-genus *Onagra* (the subgenus which has furnished almost the sole material for cyto-genetic studies so far). The various wild races are being considered from both the systematic and the cyto-genetic points of view. By comparing the conclusions reached by these alternative approaches, it is hoped that a degree of insight into species relationships and the forces which operate in species formation will be attained, such as has been rarely found possible. These joint studies are still in an incipient stage, but a preliminary cyto-genetic study of races from California by the writer (26) has led to the following conclusions based upon cyto-genetic data, some of which, at least, have been found to agree with conclusions based upon systematic studies:<sup>1</sup>

- (1) The *onagras* of California are characterized by the presence of mostly or entirely paired chromosomes, in contrast to those from other regions, almost all of which, so far studied, have shown large circles.

- (2) California races have shown no evidence of lethal factors, which are so preeminently characteristic of races from elsewhere.

- (3) California races have very little seed and pollen sterility; other *onagras* show, as a rule, high sterility.

- (4) The gene complexes or genomes composing plants of the California races are identical or at least similar, genetically; those found in other races are usually very distinct. Consequently, races from outside California may ordinarily be referred to as "complex-heterozygotes"—those from California cannot be so designated.

- (5) The various races in California and the vicinity of California resemble one another closely in the arrangement of end segments, as is shown by the fact that hybrids between them yield entirely or mostly paired chromosomes. Assuming that similarity

<sup>1</sup> The writer acknowledges with gratitude generous support from the Penrose Fund of the American Philosophical Society, which has made this study possible.

in segmental arrangement is an indication of near relationship, this fact indicates that these races are phylogenetically close to each other.

(6) Races of the California group, when crossed with races from other regions, produce hybrids with a great variety of chromosome configurations—almost all 15 of the possible chromosome arrangements that can be found in 14-chromosome individuals having been observed in these hybrids.

Most of the complexes from outside California show no very close relationship to the California group, producing large or relatively large circles with them. But curiously enough, some complexes belonging to extra-California races have proved to be essentially of the California type, having segmental arrangements of the same general type and thus giving mostly paired chromosomes when combined with California genomes. The complexes which have shown this close relationship are *velans*, *flavens*, *acuens*, *excellens* and, to a slightly lesser degree, *rigens* and *fascians*. It is interesting that this list includes the two complexes, belonging to complex-heterozygotes, which are known to be alethal or, at the most, possessed of a semi-lethal, namely, *acuens* and *flavens*. It is also interesting that *velans*, *flavens* and *acuens* are complexes which Renner had decided earlier were closely related genetically to each other and to *hookeri*, one of the California genomes.

The races which contain these 6 complexes were obtained from widely scattered regions, although there is a possibility that they were not in all cases indigenous in the regions where they were found—in fact, in the case of 2 of them, which belonged to races picked up in Europe, it is evident that they were not in their native haunts. The races picked up in America came from such widely separated regions as Illinois, Alabama and Massachusetts, a long distance, therefore, from California.

We find, therefore, that races from regions far removed from California, so far as they have been studied, have complexes which for the most part show little or no resemblance in segmental arrangement to those from California, but in some cases they possess one complex of the California type, associated with a complex which is not of the California type.

These facts raise a number of interesting questions: (1) How are we to account for the very different cyto-genetic behavior of

onagras from California and those from other regions? (2) To what extent are complexes of the California type actually distributed in the various areas outside California and how is it that they should have found their way into these regions? (3) How are the complexes which are not of the California type to be classified from the standpoint of segmental arrangement? Do they also belong to a single type, or do they fall into several categories, or are they so varied as to escape classification? (4) Is there any evidence that segmental arrangements not of the California type are as characteristic of certain other geographical areas as the California type is characteristic of the far west—in other words, is there evidence with regard to the probable origins of complexes not of the California type?

Complete answers to these and other questions must be sought through further investigation; at present the best one can do is to hold tentative opinions or working hypotheses. They indicate, however, some of the directions in which present research is moving and the sort of problems which it may be possible to solve through a combined taxonomic and cyto-genetic investigation of the genus.

#### TELOSYNAPSIS OR PARASYNAPSIS?

*Oenothera* has been considered, since the beginning of Gates' and Davis' cytological studies on the genus (39-41, 50-52), an outstanding example of the phenomenon of telosynapsis. In recent years, the position that *Oenothera* is telosynaptic has been attacked with vigor and it is now apparent that the original position requires modification.

The essential differences between the telosynaptic and parasynaptic interpretations of meiotic prophase behavior are these:

(1) According to telosynaptists, the side by side association of homologous chromosomes does not occur until late prophase ("strepsinema," "second contraction"); hence, the spireme previous to this stage (in a stage corresponding to "pachyphase") is univalent. According to the parasynaptists, synapsis occurs early in prophase and, as a result, the pachyphase spireme is bivalent.

(2) The early spireme, on the telosynaptic interpretation, is continuous and the chromosomes are united end to end until the time for side by side pairing arrives. This end to end union does

not imply homology between the attached ends. According to the parasynaptic interpretation, however, there is no continuous spireme. The chromosomes are not attached end to end, their only association in prophase being that of synapsis, which type of union does definitely imply homology between the associating parts.

All the earlier workers in *Oenothera* cytology adopted the telosynaptic point of view, chiefly for 2 reasons: (1) Splits were not visible in the stages which would correspond to pachyphase and early diplotyphase in other organisms; the spireme was thus thought to be univalent. (2) The early spireme appeared to be continuous and the chromosomes in diakinesis were mostly attached end to end to form a univalent chain. The workers adopting the telosynaptic point of view included Gates (50-52), Davis (39-41), Cleland (13-15, 17, 18), Håkansson (55, 56), Sheffield (89, 90), Illick (61, 62), Kulkarni (64, 65), Hedayetullah (58), Capinpin (8, 9), Sinotô (96).

On the other hand, certain writers have expressed skepticism of the telosynaptic point of view as applied to *Oenothera*, some of these supporting without qualification the parasynaptic interpretation. These authors included Schwemmle (88), Kihara (63), Boedijn (6, 7), Leliveld (67, 68), Darlington (35, 37, 38), Catcheside (10-12), Emerson (45), Gates and Goodwin (53), Wisniewska (108), Weier (107).

There is not space in the present article to enter into the pros and cons of this subject. Suffice it to say that the bulk of evidence seems now to support the conclusion that the chromosomes in *Oenothera* are associated side by side at least at the ends. How far back from the ends synapsis occurs is at present a matter of controversy. Darlington, strongest proponent of parasynapsis in *Oenothera*, argues for the existence of "differential segments" in the centers of the chromosomes, in which the genetic material is not necessarily arranged in the same order in the various complexes. This would effectively prevent synapsis in the interstitial segments, except in rare instances where homologous bits were so situated in opposing complexes that they could synapse. If Darlington's concept is correct, as it may well be, at least in some degree, it naturally follows that most of each chromosome in "pachyphase" is unpaired and that, therefore, a considerable portion of the thread system at this stage is univalent. A really criti-

cal piece of work has yet to be done to settle the question as to how much of the chromosome is paired and how much unpaired at this time. At present, however, we may accept the concept that chromosomes in *Oenothera* synapse at the ends, leaving the question open as to whether the central regions follow suit. If synapsis is found to be general throughout the length of the chromosome, *Oenothera* will fall into line with most other organisms in constituting a more or less typical example of parasynapsis. If, however, Darlington's contention is correct, *Oenothera* will have to be considered somewhat exceptional, for, on the one hand, it will be seen to be parasynaptic at the ends, but, on the other hand, will show for the most part the univalent spireme claimed by telosynaptists as typical of the meiotic prophase stages.

The aspects of the cyto-genetic investigation of *Oenothera* which have been reviewed in the present paper are some of those with which the reviewer is in most intimate touch. There are other important aspects which have had to be omitted for lack of space. Thus, there are the interesting embryological studies of Renner and his students; the significant contributions of Gates, Davis, Håkansson and others to our knowledge of non-disjunction, trisomy, haploidy and polyploidy in the group; as well as investigations into the nature of *Oenothera* "mutants," in general, and into the effects of environmental conditions and of radiations upon cytological and genetical behavior. A review of these aspects of the subject must await a more extended survey of *Oenothera* research.

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## GLOSSARY

- crossing-over: an exchange of parts between homologous chromosomes. It results in the separation of genes which are ordinarily linked.
- diakinesis: the last stage in the prophase of the first meiotic division.
- diplophase: the stage in prophase of the first meiotic division when the synapsed chromosomes begin to fall apart.
- fragmentation: breakage in a chromosome.
- genom: the entire set of chromosomes inherited from one parent.
- independent segregation: the segregation of maternal and paternal chromosomes of any homologous pair independently of the direction of segregation in any other pair.
- lethal (factors): factors which render inviable an organism possessing them in a homozygous condition, or (occasionally in plants) factors which prevent the functioning of gametes.
- lethals, balanced: lethal factors in opposite genomes. Individuals possessing them appear to breed true because one half of the progeny are homozygous for a lethal and perish, or because one genom fails to function as sperm, the other as egg.
- linkage: the association of genes in the same chromosome, and the consequent tendency of the characters which they govern to be inherited together.
- meiosis: the period during which homologous chromosomes, or parts of chromosomes, pair (synapse) and then separate, so that the number of chromosomes is reduced from the diploid ( $2n$ ) to the haploid ( $n$ ). It immediately precedes spore or gamete formation, and consists of two successive nuclear divisions.
- non-disjunction: the failure of homologous chromosomes to separate into different daughter nuclei at meiosis.
- pachyphase: the stage of the first meiotic prophase during which the chromosomes are closely synapsed.
- polyploidy: the condition when more than two sets of homologous chromosomes are present in an individual.
- chromosome rings or circles: chromosomes attached end to end in a ring. Such formations occur at meiosis in many *Oenotheras* and in certain other forms.
- segmental interchange: an exchange of segments between non-homologous chromosomes.
- spireme: during early stages of nuclear division, the chromosome is in the form of a thin thread or spireme.
- synapsis: pairing of homologous chromosomes during meiosis.
- translocation: transfer of material from one region of chromosome to another region of the same or different chromosome. Reciprocal translocation, or mutual exchange of material, is segmental interchange.
- trisomy: the condition in which one of the chromosomes is present in triplicate.

# THE BOTANICAL REVIEW

VOL. II

JULY, 1936

NO. 7

## LEAF DIFFERENTIATION IN ANGIOSPERMS\*

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### INTRODUCTION

The foliage leaf of angiosperms is so widely used in modern anatomical, physiological and genetical research that definite information regarding its growth and differentiation is highly desirable. An examination of modern botanical texts, however, reveals a surprising neglect of even the more general aspects of this problem although tissue differentiation in the stem and root is usually treated in considerable detail. This circumstance has prompted the present review which attempts to summarize and to correlate the most important of the recent studies on foliar histogenesis.

The task of integrating the widely scattered literature is difficult, particularly because of the disproportionate emphasis which has been placed recently upon the mode of origin of the leaf at the growing point. As a consequence, the later significant phases in foliar differentiation are often minimized or completely neglected, so that comparatively little information is available on the *entire* career of development of the various types of angiospermous leaves. Under such circumstances, the reviewer disagrees with the opinion recently expressed by Troll (65) that the "ontogenetic method" is subordinate in importance to the comparative or "typological method" in the study of the leaf. On the contrary, it would seem obvious that a sound and well balanced morphological treatment must attempt to relate the facts of development *impartially* to the form and structure of the adult organ. Schüep's (55) ideas in this connection are pertinent and timely. He states: "Our comparative morphology deals always with adult forms without proper regard to their method of origin. However, concepts which

\*I am grateful to my wife for her valuable assistance in preparing the illustrations and diagrams in this article. Thanks are also due to Professor D. R. Hoagland and H. S. Reed for reading the manuscript.

describe form should be replaced by concepts which deal with the regulation of growth."

In the following pages, the literature will be discussed according to the successive phases through which a leaf passes from inception to maturity. This method of treatment has two important advantages: (1) it serves to emphasize the essential relationship between the successive stages in leaf development, and (2) it permits, under each differentiation phase, of a brief indication of the problems requiring further investigation.

#### LEAF INITIATION AT THE GROWING POINT

The classical investigations of Hanstein (22) definitely established the fact that the angiosperm leaf originates from the outer layers of cells at the side of the shoot growing point. This principle of development has many significant implications. From a physiological standpoint it is clear that the factors determining the position, sequence and character of foliar primordia at the growing point are, in the final analysis, those which regulate the growth and division of cells in primordial meristematic tissue. In several recent papers Priestley (43, 44) has analyzed certain of the possible factors which may govern the shape, plane of division, and mode of differentiation of cells in the shoot apex and Zirkle (70) has given us valuable data on the cytology of primordial meristem and its derivatives. It is obvious, however, that at present we are merely on the fringe of a complex and involved series of problems, the solution and integration of which depend upon further knowledge of protoplasmic behavior.

From an anatomical standpoint, Schüepf (52) has advanced a useful theory to account for the emergence of foliar primordia at the shoot apex. He points out that since the intensity of cell division is approximately the same in all portions of the growing point, adjustment to the continued increase in volume is brought about by folding of the outer cell layers at definite points. These surface folds represent leaf primordia which thus appear as the inevitable outcome of the periclinal growth characteristic of primordial meristem. Schüepf's viewpoint has been adopted by Priestley (43, 44, 45) and Pottier (41) and in the reviewer's opinion deserves serious consideration in any effort to visualize the forces underlying the growth and behavior of primordial meristem in the shoot apex.

It must be realized, however, that the typical "surface folding" just described is usually accompanied by distinctive periclinal divisions in certain of the outer cell layers. Hanstein's (22) observations showed that such divisions are confined to what he termed the "periblem" or subepidermal layers of the growing point. Subsequent investigations have indicated that in some cases, at least, the internal cells of the leaf primordium originate exclusively from periclinal divisions in the outermost periblem layer which are accompanied by the anticlinal division and surface growth of the dermatogen or young epidermis. This condition occurs in *Elodea*, *Galium* and *Hippuris* (24) and in certain marine genera of the family *Potamogetonaceae* (41). In *Ceratophyllum* (26), however, the "plerome" of the leaf "arises from an outer cell of a strand of plerome cells radiating from the stem plerome to the leaf primordium."

Within recent years, however, interest in the problem of leaf initiation and differentiation in other types of angiosperms has been stimulated through a study of certain periclinal chimaeras.<sup>1</sup> Stated briefly, the growing point of a typical periclinal chimaera is composed of one or more external layers of cells, derived from one parent, and a central mass contributed by the other graft component. Thus, depending upon the number of external layers belonging to a given parent, one layered (haplochlamydous), two layered (diplochlamydous), and even three layered (triplochlamydous) periclinal chimaeras are recognized by Winkler (67) in *Solanum*. In a haplochlamydous chimaera, such as *S. tubingenense* and *S. Koelreuterianum*, the form of the leaf is only slightly influenced by the outer single-layered component since the bulk of the primordium is derived from the inner subepidermal layers of the growing point (32). In a diplochlamydous chimaera, however, it is obvious that the periclinal divisions associated with leaf initiation must occur *at first* in the third layer of the growing point, resulting in the elevation and incorporation of the two external layers in the primordium. Such is the case with the diplochlamydous *Solanum* chimaeras (28, 32) and with the *Crataegomespili* (33). The work of Krumbholz (29), likewise, indicates the possibility of such chimaeras in *Oenothera*.

<sup>1</sup>For a summary of the genetical and anatomical aspects of periclinal chimaeras, cf. Jones (27) and Winkler (67).



These studies, as well as others conducted under the stimulus of the theory of periclinal chimaeras, demonstrate a wide variation in respect to the rôle of the various meristematic layers in leaf and bud initiation. For example, Noack (39) concluded that in *Pelargonium zonale* "the whole mass of the leaf, including the petiole, arises from a single subepidermal layer of the shoot apex." A similar conclusion was reached by Schwarz (57) in his investigation of leaf development in *Plectranthus fruticosus* and *Ligustrum vulgare* and by Halmai (21) in a study of *Centaureum*. On the other hand, the investigations of Massey (37) on *Arabis albidia* and *Euonymus japonicus*, those of Kühl (30) on *Nepenthes* and Weidt's (66) study on *Heterotrichum macrodon* Planch., clearly show that the third layer of cells at the growing point is concerned in leaf formation. A somewhat intermediate condition occurs in *Hypericum uralum*, according to Zimmerman's (69) detailed observations, in that derivatives of the third layer form only the lower portion of the midrib while the remaining internal leaf tissue arises from the subepidermal layer.<sup>2</sup> Less attention seems to have been devoted to the situation in monocotyledons but the available data likewise indicate considerable variation in this group. Rösler (48), for example, found that the leaf of *Triticum vulgare* arises exclusively from the outermost cell layer or dermatogen, while Priestley, Scott and Gillett (46) state that in *Alstroemeria aurantiaca* Don., "the first appearance of a new primordium is a slight upfold of the superficial tissues, due in part to periclinal divisions in the dermatogen layer as well as in the underlying layers."

On the basis of the above studies, it is obviously impossible to generalize as to the most common scheme of leaf initiation in the angiosperms (6). On the contrary, it seems clear from Schmidt's (50) careful investigations that the mode of leaf formation is directly related to the architecture of the growing point in each particular case. According to Schmidt, the shoot apex consists of one or more self-perpetuating peripheral layers of cells which he collectively terms the *tunica*; periclinal divisions normally appear in certain of these layers only during leaf or axillary bud formation. The inner core of the growing point, in which growth in volume predominates, he designates as the *corpus*.<sup>3</sup> Schmidt em-

<sup>2</sup> A similar condition prevails in *H. acutum* and *H. montanum*, according to Herbst (23).

<sup>3</sup> Schmidt's terminology has been widely adopted (12, 20, 21, 30, 34, 48, 69) and in the reviewer's opinion deserves general acceptance by anatomists.

phasizes that the extent to which tunica and corpus participate in leaf or bud initiation depends upon the quantitative relationships of these regions in a given shoot apex. Thus, in *Scrophularia nodosa* L. the tunica is represented by a single layer corresponding to the dermatogen and the leaf arises from the outer portion of the corpus. In contrast, the growing point of *Vinca minor* L. possesses a three layered tunica from the inner layers of which the leaf originates. An intermediate condition is illustrated by *Veronica myrtifolia* Sm. where both tunica and corpus derivatives form the leaf initial. This latter condition is similar to Foster's (12) description of bud scale and foliage leaf emergence in *Carya* (fig. 1) and to the origin of the leaf in *Oenothera* (29).

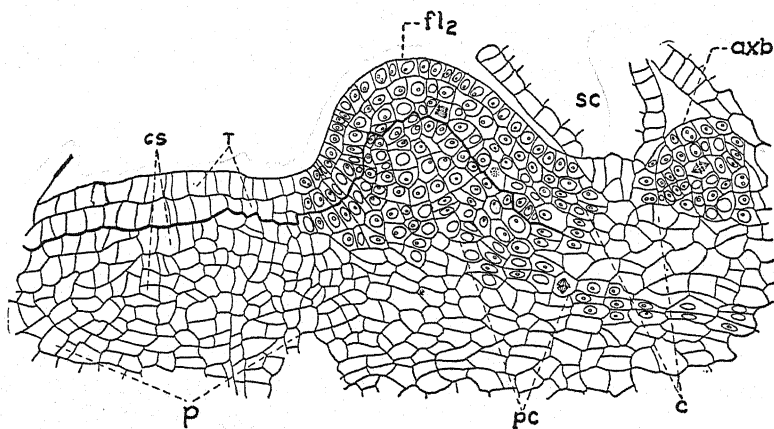


FIG. 1. (From Foster (12)). Median longitudinal section through the growing point of *Carya Buckleyi* var. *arkansana*, showing an early developmental stage of a foliage leaf. Note that derivatives of both the tunica (*t*) and corpus (*cs*) are present in the primordium. Legend: *axb*, primordium of staminate catkin bud; *c*, cortex of internode; *fl*, foliage leaf primordium; *p*, pith of stem; *pc*, procambial tissue; *sc*, portion of bud scale. ( $\times 900$ ).

#### ORIGIN AND EARLY DIFFERENTIATION OF PROCAMBIUM

For a short time after its emergence from the growing point, a foliar primordium consists of actively dividing primordial meristem cells. One of the first significant indications of specific differentiation following this phase consists in the formation of the median procambial strand near its base (fig. 1). According to Yarbrough (68), this occurs in *Bryophyllum calycinum* when the primordium "is between 60 and 70  $\mu$  long." In *Alstroemeria*,

Priestley, Scott and Gillett (46) found both median and lateral procambial strands in a primordium only  $54\ \mu$  long.

The general importance of this early appearance of procambial tissue has been emphasized by several investigators. Priestley and Scott (45) maintain that procambial cells "probably exercise a very great influence on further development and their presence is probably responsible for the fact that the leaf primordium now grows much more rapidly than the meristematic apex." The most comprehensive study of the morphogenesis of procambial tissue in the shoot apex, however, has recently been made by Louis (34) who has described and profusely illustrated the condition in various types of angiosperms as well as in *Taxus*, a gymnosperm. Confirming the earlier ideas of Gregoire (18, 19), Louis finds that the actual emergence of each foliar primordium is preceded by the transverse expansion of the shoot apex to form two (decussate-leaved plants) or one (alternate-leaved plants) axial portions which he designates as "foliar buttresses" ("soubassements foliaires"). Each foliar buttress with its vertically emergent primor-

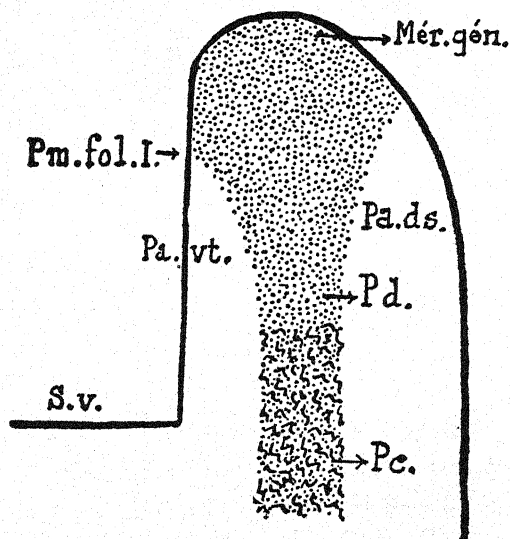


FIG. 2. (From Louis (34)). Schematic reconstruction of a median longitudinal section of a leaf primordium of *Syringa vulgaris*, showing the differentiation of procambium from prodesmogen. Legend: *mér. gén.*, general meristem; *Pa. ds.*, dorsal parenchyma; *Pa. vt.*, ventral parenchyma; *Pc.*, procambium; *Pd.*, prodesmogen; *Pm. fol I.*, leaf primordium; *S. V.*, growing point.

dium is thus regarded as a unit, an idea quite comparable to Priestley's (44, 46) "growth units" which consist of a leaf initial and a segment of the shoot axis (*cf.* also Schüepf (56)). According to Louis, the early dorsal and ventral vacuolation of cells in the lower portion of an elongating primordium results in the demarcation of a central "arc" of meristem which he terms the "prodesmogen." As figure 2 shows, the first true procambium subsequently originates from this prodesmogen. Since the further differentiation of procambium proceeds both acropetally into the primordium and basipetally into the foliar buttress, vertical "leaf traces" are produced. Thus, the earliest formed procambium in the stem consists of discrete strands (separated by undifferentiated prodesmogen) which arise in connection with the foliar buttresses and their emergent primordia. The number of strands present in each case is directly related to the phyllotaxis and the relative width of insertion of the primordia. In the reviewer's opinion, the data and ideas presented by Louis provide a basis for a truly objective study of shoot organization in the angiosperms. It seems clear that any effort to isolate the leaf or stem, at least from a morphogenetic standpoint, is unsupported by the facts of development.

#### ORIGIN OF PETIOLE, MIDRIB, AND LAMINA

Soon after its appearance at the growing point, the pad-like or peg-shaped leaf primordium embarks upon a definite career of morphological differentiation, which consists in the initiation of such parts as the petiole, lamina, sheath, stipules, etc. Thus, the basic architecture of the adult leaf originates very early in ontogeny, a fact of considerable significance to the general problem of form determination (11, 12). Early studies of leaf development, such as those of Trécul (62), Eichler (8), Goebel (15), Deinema (7) and Massart (36), were concerned largely with the *external aspects* of morphological specialization and neglected the accompanying process of internal cellular differentiation in the primordium. Unfortunately, a similar criticism can be applied to much of the modern work on leaf development. Troll (63, 64), for example, has very recently discussed in great detail the comparative morphology and form development of the peltate and pinnate type of leaf although he entirely ignores the histogenetic implications of his data. As a result of the dominance of this one-sided atti-

tude, our knowledge of the cellular differentiation of the various morphological regions of a leaf is limited to a small number of recent investigations, the results of which will now be summarized.

The available data clearly show that in many dicotyledons the formation of the lamina is preceded by the differentiation of the future petiolar-midrib region. The latter arises as the result of the early growth in length of the primordium which often acquires at this stage the form of a tapering, adaxially-flattened cone. Certain phases in the differentiation of the petiolar-midrib region have been studied by several investigators, particularly with reference to the processes of apical growth. In *Pelargonium* (39) and in *Plectranthus* and *Ligustrum* (57) apical growth is related to the continued periclinal and anticlinal division of subepidermal initials; the latter are lineal descendants of the subepidermal cell-layer of the growing point. A similar condition, according to Avery (2), occurs in the tobacco leaf where "the addition of cells at its apex may be traced to the activity of a single subepidermal cell." Other investigators, however, maintain that apical growth is not necessarily limited to the division of subepidermal initials at the leaf apex. Krumbholz (29) found that in *Oenothera* active cell divisions occur both apically and subapically, a view supported by Lange's (32, 33) detailed analysis of leaf development in *Solanum chimaeras* and in the *Crataegomespili*. It will be recalled that in the examples just mentioned, more than two layers of cells are involved in the initiation of the leaf. In all such cases, as Lange (32) emphasizes, the degree to which the derivatives of the various subepidermal layers of the shoot apex participate in apical growth varies considerably and may be traced with confidence only in certain diplochlamydous chimaeras. Similarly, Foster (12) has shown that at an early stage in the differentiation of bud scale and foliage leaf in *Carya*, no clear demarcation can be made between the derivatives of the inner layer of the tunica and the corpus in the apical region of the primordia. We may, therefore, conclude that the process of cell division in the leaf apex of angiosperms follows no universal scheme. On the contrary, true apical growth ceases relatively early and the subsequent intercalary extension of the petiolar-midrib region is accompanied by general cell division and cell extension, regardless of the histogenetic mode of origin of the leaf (2, 12, 25, 60, 66, 68).

The apical and intercalary extension of the petiolar-midrib region is accompanied in many cases by a characteristic increase in radial thickness. This type of growth is associated particularly with the activity of a vertical strip of periclinally dividing cells found beneath the adaxial epidermis. As a result of a broad comparative study, Bouygues (4) found that this type of cambial-like thickening is typical of the early phases of petiole development in many types of dicotyledonous leaves. Schüepp (53), likewise, has pointed out that in *Acer pseudoplatanus* L. the petiole arises, not as the primary result of the intercalary elongation of a zone interpolated between lamina and leaf base,<sup>4</sup> but because of a growth in thickness "involving almost the entire inner surface of the leaf base." McCoy (35) also states that the petiole of the leaf of *Zeugites* "thickens by an extended period of secondary cell division in its ground tissue," a situation which is duplicated in the leaves of certain aroids (7). In various species of *Carya* (12, 13) the early marked increase in radial thickness due to this adaxial strip of meristem produces a massive leaf-axis or phyllopodium (5) which later specializes into the leaf-base, petiole and rachis of the pinnate leaf; a similar type of meristematic activity also occurs in the midrib of each leaflet (fig. 3).

Following the early specialization of the petiolar-midrib region of most simple leaves, the lamina begins to differentiate from its upper portion as two thin marginal ridges of meristem (fig. 3). The stage at which laminar differentiation begins varies considerably. Marginal growth in *Oenothera* (29) and in *Plectranthus* (57) occurs when the primordium is less than .1 mm. in height, and in *Bryophyllum* it "is evident as early as the 350  $\mu$  stage" (68). In *Nicotiana* (2), however, the lamina does not appear until the embryonic midrib is approximately .6 mm. long. As in the case of apical growth, divergent ideas exist as to the importance of the subepidermal layer of the midrib in the origin and marginal growth of the young lamina. Gidon (14), Noack (39), Schwarz (57), Avery (2), Johnson (25) and Weidt (66) maintain that the differentiation of the lamina may be traced to the activity of a band of submarginal cells at the edge of the midrib. A similar condition appears to explain the origin and early growth of the lamina from

<sup>4</sup> This interpretation of petiole development originated with Eichler (8) and dominates Goebel's (16) viewpoint of foliar ontogeny.

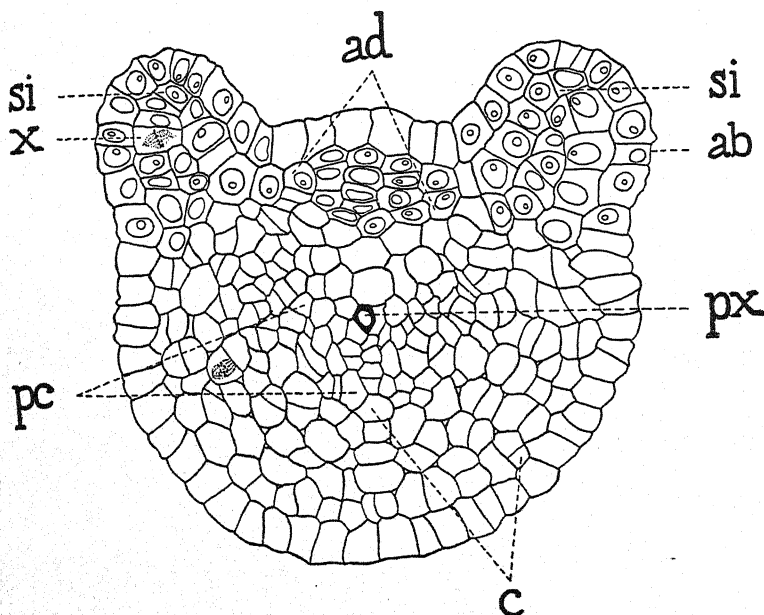


FIG. 3. Transverse section near base of median leaflet (224  $\mu$  high) of a young seedling foliage leaf of *Carya Buckleyi* var. *arkansana*, showing the origin of the lamina from the adaxial margins of the midrib. Note that each lamina half consists of three internal layers of meristematic cells enveloped by the epidermis. The periclinal division in cell X illustrates the point of origin of the middle layer. Legend: *ad*, adaxial meristem of midrib; *ab*, abaxial surface of lamina half; *c*, cortex of midrib; *pc*, procambium; *px*, protoxylem element; *si*, submarginal initial of lamina. ( $\times 900$ ).

the leaflet-midribs in various species of *Carya* (12, 13, 31). Krumbholz (29) and Lange (32), while admitting the importance of such localized marginal growth, contend that both subepidermal, as well as deeper, cell layers may participate to varying degrees in lamina formation (cf. also K  hl (30)). Additional studies on the complete developmental history of the leaf in a wide range of angiosperms are obviously needed before any general conclusions can be reached. As an indication of the necessity for more data may be cited the recent observations of Renner (47) on lamina differentiation in a white-margined haplochlamydous chimaera of *Sambucus nigra*. According to Renner, the edge of the young lamina is provided with a strip of periclinally-dividing dermatogen cells, which contribute to the colorless marginal region of the leaf. Although periclinal divisions in the marginal dermatogen cells occur during

lamina differentiation in certain monocotyledons (41), Renner is one of the first to describe a similar situation for a dicotyledon. A thorough study of both normal and periclinally-variegated leaves in dicotyledons would possibly bring to light additional examples of this condition.

The differentiation of petiole, midrib and lamina described above appears characteristic of many simple leaves and corresponds to what Prantl (42) termed the "pleuroplastic type" of development. Almost no information exists, however, regarding the cellular differentiation of pinnate, digitate (*i.e.*, "palmate") or pedate leaf types, in spite of their widespread occurrence in dicotyledons. According to Troll's (64) recent monograph, the leaflets (or lobes) of such leaves may arise in three possible ways; *viz.*: (a) in *basipetal sequence*; *i.e.*, from the apex towards the base, (b) in *acropetal sequence*; *i.e.*, from the base towards the apex, and (c) in *divergent sequence*; *i.e.*, the first pair of leaflet primordia arises near the middle region from which point additional pinnae develop both acropetally and basipetally. The "basipetal leaf" seems to dominate in the angiosperms and represents, in Troll's (64) opinion, the "basic type" to which may be traced the manifold character of pinnate leaves (*cf.* also Troll (65)). The differentiation of the typical acropetal leaf of *Carya* has been described by Foster (12, 13) who finds that the lateral leaflet initials arise as hemispherical protuberances from the primordial meristem at the margins of the leaf axis, the apex of which produces the terminal leaflet (*fig. 4*). As far as the reviewer is aware, however, no similar study has been made of either a basipetal or divergent type of pinnate leaf. The problem clearly requires further histogenetic investigation, especially since Troll states that the basipetal and acropetal order of leaflet formation is usually correlated respectively with an early or late cessation of apical growth in the primordium as a whole. With this idea as a basis, it should be possible to connect the histogenetic process with the elaboration of form in the lamina.

#### HISTOGENESIS AND GROWTH OF THE LAMINA

Recent investigations on the origin and differentiation of tissues in the lamina have raised many points of considerable anatomical, physiological and genetical interest.<sup>5</sup> The most important results

<sup>5</sup> The older, scattered literature in this field has been discussed by Avery (2) and Smith (59).





FIG. 4. (From Foster (12)). Longitudinal section through a foliage leaf (288  $\mu$  high) of *Carya Buckleyi* var. *arkansana*, illustrating the acropetal formation of the lateral leaflet primordia. Legend: c, cortex of leaf axis; h, hair;  $ll_1$ - $ll_5$ , primordia of lateral leaflets;  $tl$ , terminal leaflet. ( $\times 900$ ).

of these studies may be conveniently summarized under three main topics:

1. *Marginal growth.* Smith (59) has shown in a recent comparative survey that the embryonic lamina consists of 5-8 distinct layers of meristematic cells which ultimately differentiate into the epidermis, mesophyll and vascular bundles of the adult leaf blade. The origin and behavior of the typical stratified meristem of the young lamina and its relation to the adult "tissue pattern" have been studied by other investigators in a number of species and the data are presented schematically in figures 6 and 10. In all these cases, a distinctive type of "marginal meristem" is present, from which the various embryonic layers originate. The outermost cells of this meristem represent the "marginal initials" (Mi in all figures) and give rise to the upper and lower dermatogen layers which perpetuate themselves by anticlinal divisions and surface growth. Similarly, the continued formation of the internal layers may be traced to the activity of a band of "submarginal initials." (Si in all figures). The diagrams, however, clearly indicate two distinct types of behavior of the submarginal initials. In one type, as illustrated by *Bougainvillea* (14), *Pelargonium* (39), *Plectranthus* and *Ligustrum* (57), *Nicotiana* (2), and *Kalanchoë* (25), the sub-

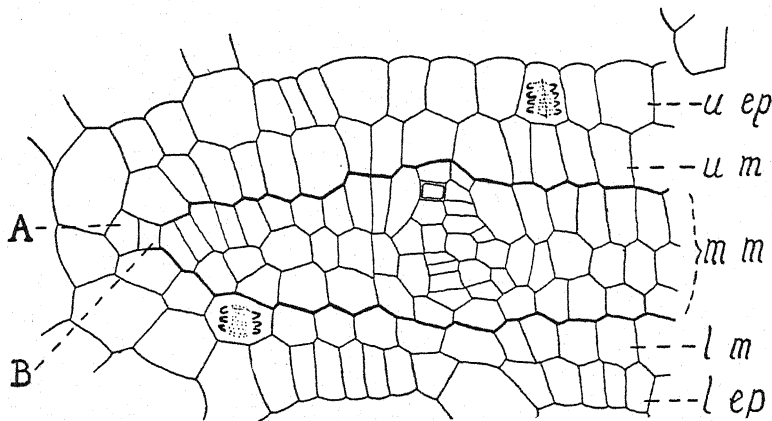


FIG. 5. (From Avery (2)). Transverse section of outer edge of young lamina of *Nicotiana tabacum*, showing anticlinal division of submarginal cell into cells A and B, and the genesis of the internal layers. Legend: *u ep*, upper epidermis; *u m*, upper or adaxial mesophyll layer; *m m*, middle mesophyll layers with young vein; *l m*, lower or abaxial mesophyll layer; *l ep*, lower epidermis.

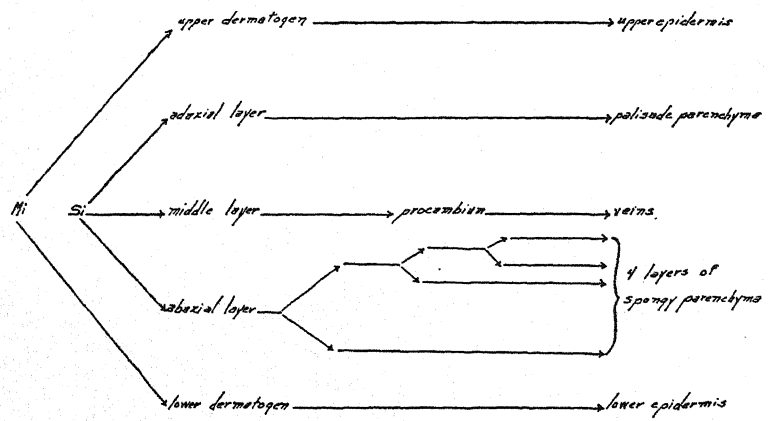


FIG. 6. Schematic representation of tissue differentiation in the lamina of *Bougainvillea spectabilis*. (Based on Gidon (14)).

marginal initial divides at right angles to the surface of the blade, forming an outer and an inner cell (cells A and B of fig. 5). From the inner cell (B) the middle layer or layers of the lamina are derived. The outer cell (A) meanwhile divides parallel to the leaf surface, one daughter cell persisting as an initial, the other being added either to the abaxial or adaxial layer. Thus in this type, anticlinal and periclinal division-planes alternate in the sub-marginal initial and the origin of the main internal layers may be traced to the very edge of the lamina (fig. 5). According to Avery

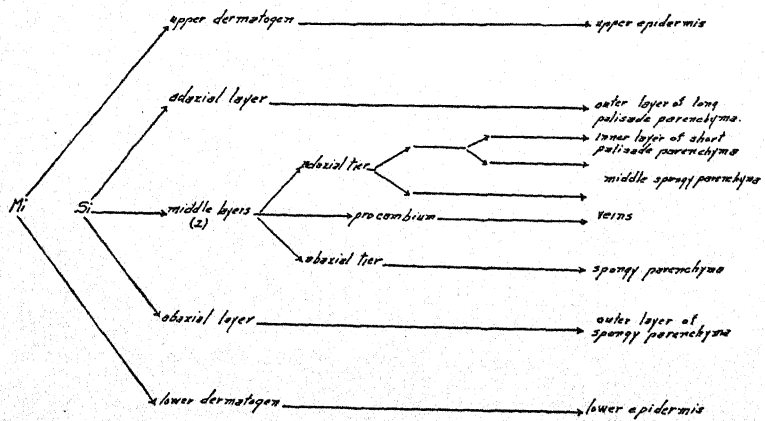


FIG. 7. Schematic representation of tissue differentiation in the lamina of *Pelargonium zonale*. (Based on Noack (39)).

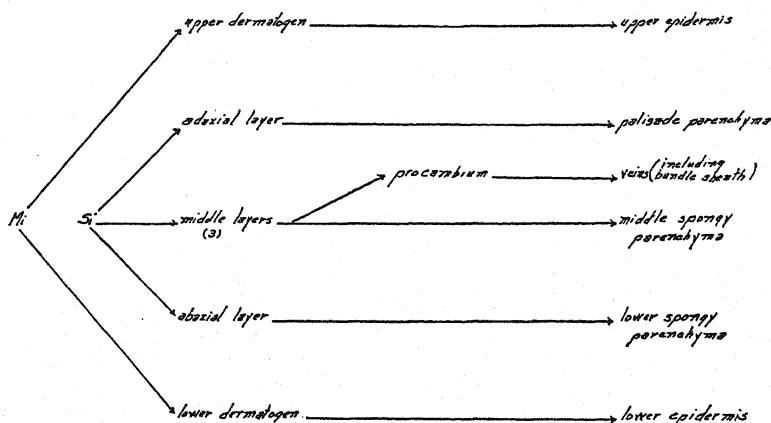


FIG. 8. Schematic representation of tissue differentiation in the lamina of *Nicotiana tobacum*. (Based on Avery (2)).

(2), this characteristic behavior of the submarginal initials in *Nicotiana* "apparently continues as long as there is marginal growth." A contrasted type of submarginal activity is illustrated by *Carya* (12, 13) and by two species of the Melastomaceae; viz., *Heterotrichum macrodon* Planch and *Clidemia hirta* Don. (66). Here the submarginal initials, by alternating oblique divisions, first produce two internal layers of cells (figs. 9-10). At a varying distance from the leaf margin, the abaxial layer divides periclinally, thus producing an inner or middle tier of cells (cf. fig. 3).

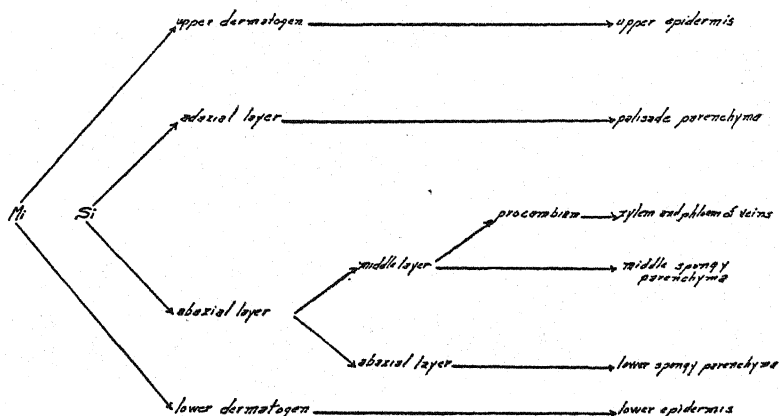


FIG. 9. Schematic representation of tissue differentiation in the lamina of *Carya Buckleyi* var. *arkansana*. (Based on Foster (12)).

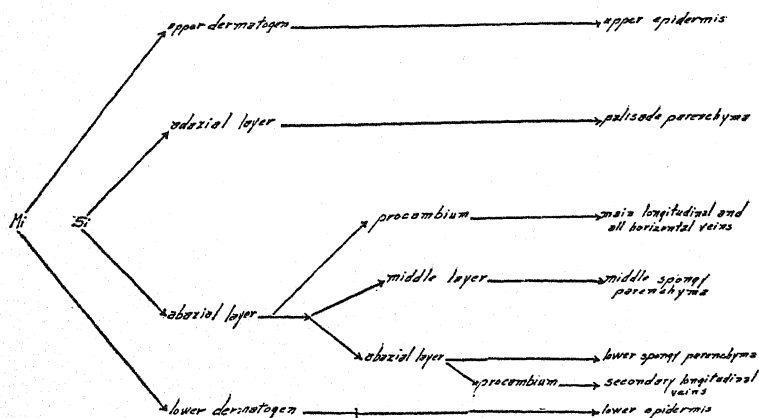


FIG. 10. Schematic representation of tissue differentiation in the lamina of *Heterotrichum macrodon*. (Based on Weidt (66)).

The distribution of these two distinct types of marginal growth in the angiosperms deserves careful investigation. That other modes of lamina formation exist is indicated by Skutch's (58) observations on the leaf of *Musa sapientum* L. Here the initiation of the lamina-halves occurs at a relatively late stage "when the marginal cells of the midrib have lost their meristematic nature and have differentiated into a scarious border." Thus in *Musa*, lamina differentiation is relegated to deeper layers and a strictly marginal meristem is absent.

2. *Surface growth and the regulation of form.* True marginal growth in the lamina is of short duration as compared with the protracted surface growth characteristic of later phases in development. This is clearly shown in the "basipetal type" of leaf where the cessation of marginal activity proceeds from the apex towards the base. Thus in *Pelargonium*, marginal growth ceases when the main lobes of the lamina have been formed (39). A similar early maturation of the marginal meristem likewise obtains in other basipetal types (66), so that all further growth in the area of the lamina is due largely to cell division and cell extension throughout the embryonic layers, to which Schüepf (52, 54) has given the collective term of "plate meristem." Cell division in the various layers of the plate meristem is predominantly anticlinal in respect to the surface of the lamina, with the result that the characteristic number of layers established by marginal growth is relatively constant

throughout the development of the leaf (2, 12, 51, 66). The only important disturbance in the typical stratified appearance of the plate meristem during surface growth results from the early differentiation of procambial strands in certain of the layers (fig. 5). However, the areas between procambial strands or even well-differentiated vascular bundles remain undifferentiated and capable of prolonged growth (12). As Schüepf (51) has shown in *Acer pseudoplatanus* L., the characteristic plicate veneration of the lamina in the bud is related to the adjustment between surface growth and the longitudinal extension of the prominent ribs of the main veins.

It is extremely difficult to visualize the complex relation of marginal and surface growth to the adult form of the lamina. Tetley (61) suggests that "the balance between the rate of differentiation and the capacity for extension of the marginal cells on the one hand, and the rate of differentiation of the mesophyll within is evidently an important factor in determining the size and shape of the leaf." Avery (2) has approached the problem of leaf form in *Nicotiana* from the standpoint of the relation of differential rates of growth in various parts (or "segments") of a developing lamina to the growth of the leaf as a whole. The ratio between the relative growth of any measured segment of the leaf and the total area of the blade during various stages in development, is expressed mathematically by the symbol " $k$ ." Avery has determined the values for  $k$  throughout the growth of the leaf and concludes from his data that "localized growth" (i.e., differential distribution of growth in various portions) and "polarized growth" (i.e., greater growth in one dimension than in another) are responsible for the final shape of the organ. More recently, Avery (3) has investigated the role of auxin  $a$  in the growth and differentiation of the tobacco leaf. His data suggest that auxin may be partly responsible for polarized growth although the effect of this hormone on localized growth remains to be investigated. Avery's work clearly indicates the need for a careful examination of the implications of modern hormone research on developmental problems. In addition to hormones, the relation of certain microelements, such as copper, zinc and manganese, to leaf growth deserves investigation. Obviously, in view of the great variation in the distribution of growth in the lamina of dicotyledons (40, 42), the whole subject challenges anatomical and physiological technique.

3. *Differentiation and maturation of tissues.* The destiny or "prospective significance" of the internal layers<sup>6</sup> of the young lamina represents a problem of considerable morphogenetic interest which can be reviewed only briefly in this paper.

Most investigators agree that the procambial strands, destined to form the xylem and phloem of the veins, arise from the middle cell layer or layers of the lamina (cf. fig. 5). Although, as Smith (59) maintains, this may be the usual condition, several interesting exceptions deserve mention. Mounts (38) found that the veins in the leaf of *Vitis* and *Catalpa* arise from the uppermost or adaxial layer while Weidt (66) describes two distinct methods of origin of procambial strands in *Heterotrichum* (fig. 10). In general, if supporting tissue (collenchyma or fibers) accompanies the veins, it originates from the abaxial and adaxial subepidermal layers (12).

In view of the wide variation in the number, position and character of the cell layers in the mesophyll of angiosperm leaves, no uniform scheme of origin of this tissue region may be anticipated. However, reference to figs. 6, 8, 9, 10 will show that the palisade parenchyma, at least in the dorsiventral type of blade, usually arises directly from the adaxial subepidermal layer. According to Avery's (2) observations, cells of the palisade layer appear distinct in size "almost as soon as the lamina is initiated." In some cases the spongy parenchyma of the leaf may originate from the abaxial layer as well as from the non-vascular areas in the middle layers of cells (figs. 7-10). In other plants, such as *Bougainvillea*, the spongy parenchyma is formed by repeated periclinal division of cells derived from the original abaxial layer (fig. 6). As far as the writer is aware, no attention has been paid to the genesis of the mesophyll in the isolateral type of leaf, although information on this point would bear directly on various problems of "ecological anatomy."

In conclusion, brief mention should be made of certain aspects of tissue maturation which are of general physiological and anatomical interest. Recent studies have clearly shown, in the first place, that cell division and cell enlargement are unequal in duration and intensity in the various tissues of a maturing leaf. For example, the cells of the palisade parenchyma continue to divide for a longer period than either the spongy parenchyma or the epidermis (2, 38,

<sup>6</sup> The upper and lower external layers are omitted from discussion since they invariably produce the epidermal system.

66). On the other hand, cell enlargement continues longer in the epidermis than in any other tissue (2, 38, 68). As a result of this inequality in growth of the various layers, it is assumed that "stresses" and "strains" arise which are responsible for the pulling apart of the subepidermal cells and the formation of the physiologically important air-spaces of the mesophyll. Mounts (38) concludes that "insufficient stress has heretofore been placed upon the mechanico-dynamic function of the epidermis" which appears to be "a major factor in leaf expansion." Avery (2) adopts a similar viewpoint, and in addition suggests that the wavy contour of the cells of the lower epidermis is produced by the reciprocal "pull" imposed upon them as the cells of the spongy parenchyma become separated. Further investigations, however, are urgently needed before we can view the complex process of tissue maturation entirely from such a "mechanical" viewpoint.

#### FOLIAR DETERMINATION IN ANGIOSPERMS

Although such diverse organs as bud scales, bracts and floral appendages have long been regarded as the homologues of "leaves," little detailed information is available regarding their mode of origin or cellular differentiation. The writer (9, 10, 11, 12, 13) has recently discussed this question and has applied the phrase "foliar determination" to the complex series of processes (cellular, genetic and physiological) which regulate the production of various foliar structures at the shoot apex. It must be admitted that we possess virtually no knowledge of the interaction of genetic and physiological factors during foliar differentiation. Although every cell at the growing point may be "omnipotential," the "prospective value" of the various cells and layers is doubtless limited by such factors as their relative position, nutrition, etc. (32, 49). An experimental attack on those problems is needed but great difficulty arises, with our present technique, because of the small size and delicate nature of the shoot growing point.

In spite of the difficulties inherent in the determination problem in plants, a survey of the comparative histogenesis of various types of foliar structures is first of all desirable. Several recent contributions to this aspect of the question deserve brief mention here. Schüepp (53) concludes, as a result of his experimental-developmental study on *Acer pseudoplatanus* L., that the "determination"



of bud scale or foliage leaf coincides with the emergence of the primordium at the growing point. He further indicates the importance of the type, position and duration of meristematic tissue in regulating the form and size of the adult organ. Schüepf's viewpoint has been adopted by Foster (12, 13) in his detailed investigation of bud scale and foliage leaf histogenesis in various species of *Carya*. In *C. Buckleyi* var. *arkansana* Sarg. a histogenetic divergence between scale and foliage leaf occurs when their respective primordia are less than .1 mm. in height. From this point, each foliar type embarks upon a specific and dissimilar career of differentiation. The "transition forms" likewise experience an early and specific differentiation characterized by the combination of histogenetic processes "normally confined, respectively, to the bud scale and foliage leaf."

Finally, attention must be drawn to the recent histogenetic studies on the flower by Grégoire (17, 20). He finds that in *Aquilegia*, *Ranunculus* and *Magnolia*, for example, the young receptacle consists of a massive parenchymatous core enveloped both apically and laterally by several layers of "germinative meristem," from which arise the primordia of petals, stamens and carpels. Thus, Grégoire argues, vegetative and floral "growing points" are fundamentally different in structure and the generally assumed homology between petals, stamens and carpels, and the foliage leaf does not exist.<sup>7</sup> Although Grégoire's general viewpoint has been rejected by Troll (65), his observations are of interest and similar studies in a wide series of different floral types should be made. It seems only proper to conclude that a well-balanced interpretation of foliar structures in the angiosperms must consider and incorporate the evidence furnished by developmental studies (1, 10, 55).

#### EXPLANATORY NOTES

*By the Editors*

abaxial: the side of a lateral organ away from the axis.

acropetal: produced in a succession towards the apex.

<sup>7</sup> Grégoire further develops this thesis in a more recent article, "Sporophylles et organes floraux, tige et axe floral" (Rec. Trav. Bot. Néerl. 32: 453-466. 1935), which has just come to the writer's attention. Particular emphasis is placed upon (a) the origin and early histogenesis of stamens and carpels and (b) the divergence in mode of vascularization and the "dishomology" between a leafy shoot and a flower. Grégoire rejects the classical interpretation that floral organs are sporophylls and concludes that stamens and carpels, respectively, are "microsporangio-phores" and "spermatophores."

- adaxial: the side of a lateral organ facing the axis.  
 anticlinal: perpendicular to the surface.  
 auxin *a*: one of the three growth-promoting chemical substances which have been isolated in crystalline form from plants. See Went's article in Bot. Rev. 1: 162-192. 1935.  
 basipetal: produced in a succession towards the base.  
 chimaera: the product from a bud involving mechanical coalescence of two different parental tissues.  
 histogenesis: the study of the origin and formation of tissues.  
 homologous: parts which are similar in origin regardless of their form or function.  
 periclinal: parallel to the surface.  
 periclinal chimaera: a graft-hybrid.  
 periblem: the cylinder of very young cells just inside the epidermis of a growing point, enclosing the plerome and developing into the cortex.  
 plerome: the central core of very young cells within the periblem at a growing point of roots and stems. It develops into the vascular and other tissues.  
 phyllotaxy (is): arrangement of leaves with respect to the axis.  
 procambial strands: strands of elongated cells which develop into food- and water-conducting cells.

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## PLANTS MADE POISONOUS BY SELENIUM ABSORBED FROM THE SOIL

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It was rather alarming to read four years ago that grain from certain sections of the great cereal-growing region of the north central plains harbors the cause of a serious and sometimes fatal disease afflicting farm animals and possibly also man; that farm animals get the disease from eating hay and grain raised in the affected areas; and that flour and other mill products made from the grains might poison human beings (1). Equally startling was the statement that records of similar disorders have been found running back for many years, into early exploration days, but that until recently the malady was merely shoved aside as "alkali trouble" and not seriously considered (1). A review of the literature shows that a number of phases of this problem are being studied. Much is yet to be discovered, however, and many interesting problems remain to be solved by workers in botany, chemistry, veterinary science, public health, and government.

### HISTORY

For many years a disease of livestock, including poultry, has been known west of the Missouri River in South Dakota and adjacent areas, and has been referred to as "alkali" disease, because of a relationship believed to exist between the alkali in the water or soil and the development of the disease. Apparently the earliest account of the malady is to be found in a report written in 1860 by Madison (39). Madison described the symptoms of a fatal disease which affected the cavalry horses, Indian ponies, and mules at Fort Randall, then in the Territory of Nebraska but now included in South Dakota. He attributed the disease to the pasturage. A recent study of the soils and plants of this area has shown that the conditions have not changed materially in the 80 years that have elapsed since Madison's observations (9).

The third annual report of the Wyoming Agricultural College and Experiment Station in 1893 alluded to a peculiar disease of horses in Carbon County, Wyoming (cf. 12). The symptoms were

typical of alkali disease, although at that time ascribed to ergot poisoning from hay.

Peters (44) in 1904 gave an accurate description of alkali disease in Boyd County, Nebraska, where it had been prevalent among all kinds of livestock since the settlement of that region in 1891. Corn from the affected farms was shipped to the Nebraska Experiment Station, where it was fed to hogs. In a short time it produced symptoms identical with those observed on the farms in Boyd County. Although Peters was apparently in error in concluding that only moldy corn was culpable, he reported testimony of farmers which incriminated sound corn, other grains, and pasture.

Farmers in affected areas early learned to associate the disease with particular and often rather restricted tracts of land. Many of them believed that the disease was caused by something in the vegetation. A few continued to think that the toxic effects were due to alkali in the water which the animals received. But careful investigation in 1912 and 1913 served to eliminate the water as the cause (34, 35).

Lipp (37) in 1922 gave a brief but well written account of alkali disease in South Dakota west of the Missouri River. He stated that farmers living in the alkali areas were firmly convinced that the growing forage plants store a sufficient quantity of the alkalis to cause the disease. Dealers in grain and hay, he said, refused to purchase products raised in certain restricted areas. The only sure means of prevention consists in the use of feeds grown on areas where the disease is unknown.

Byers (9) gives an interesting historical account of the recent scientific study that led to the discovery of the cause of the disease. In 1928 Dr. K. W. Franke, of the South Dakota Experiment Station, began his important series of investigations which have definitely proved that alkali disease is produced by consumption of grain, forage, and fodder grown upon definite soil areas. Early in 1931 a cooperative study of the problem was begun, which involved work in the bureaus of Home Economics, Animal Industry, Plant Industry, and Chemistry and Soils. In May, 1931, at a meeting of the interbureau committee Dr. H. G. Knight suggested that selenium be looked for in the grain. Dr. W. O. Robinson was furnished a sample of toxic wheat. He analyzed this and found that

it contained from 10 to 12 p.p.m. (parts per million) of selenium (47). Selenium was also present in soils from the affected areas.

A preliminary field survey of the alkali disease of livestock was made in 1931 by Franke and others (27). The survey included the central and southwestern parts of South Dakota, parts of northern Nebraska, and the eastern edge of Wyoming. The toxicity of the grains and grasses was found to be correlated with soils derived from Pierre shale. The disease occurs over the entire area of these soils, but is not evenly distributed. It seems to be absent in some areas comprising many square miles. There also seem to be seasonal variations. Where the disease is severe, considerable livestock may die or have to be killed, and eggs do not hatch. In such localities the raising of hogs, horses, poultry, and even cattle has been discontinued, and tractor farming of small grain is the sole substitute. Grain known to have been produced on affected soil brings only minimum prices. Likewise, affected animals usually bring low prices when marketed. Owners of some badly affected farms have abandoned them, not being able to finance, lease, or sell them. In many cases, new renters, unfamiliar with the conditions, have had heavy losses within a year after moving to such farms.

In Wyoming the injury inflicted through poisonous vegetation is most serious on the ranges, and in some districts this injury has prevented profitable use of lands for grazing (4, 5, 6).

It is of historical interest that Crawford (11) in 1908 was probably the first person to suggest that certain obscure chronic diseases of livestock on our unirrigated plains might be caused by inorganic constituents of the forage plants. He called attention to another significant point—namely, that some “loco” plants, though usually toxic, were non-toxic when grown on certain soils. He believed that the inorganic constituents were responsible for the poisonous action of the “loco” plants *Astragalus mollissimus* and *Aragalus lamberti*, at least those collected at Hugo, Colo.—a region which Byers (9) has recently shown to be seleniferous. Crawford seems to have been incorrect, however, when he concluded that barium was the poisonous inorganic constituent of the plants.

#### CAUSE AND NATURE OF THE DISEASE

*Alkali disease.* In horses, cattle, and swine alkali disease manifests itself by a depressed rate of growth, a loss of hair (conspicu-



ous in the case of the mane and tail of horses), and an abnormal development of hoofs, followed in severe cases by the sloughing off of the old hoofs (27, 37, 44). The most severely alkali-d animals die or have to be destroyed. In poultry the malady results in non-hatchability of eggs. The few young that hatch are weak and have a greased appearance.

*Selenium in wheat and soil.* The first indication as to the nature of the toxic principle in cereals was published in 1933 by Robinson (47). He reported the occurrence of 10 to 12 p.p.m. of selenium in wheat and of 0.3 p.p.m. of selenium in soil from affected areas. Byers (8) found selenium, chromium, vanadium, and arsenic in soil from the same regions. In wheat from this locality he found selenium and vanadium.

*Toxicity of plants containing selenium.* Nelson, Hurd-Karrer, and Robinson (41) reported that plants absorb selenium from the soil, and become highly toxic to animals. Quantities of selenium as small as 1 p.p.m. in the soil, added as sodium selenate, permitted growth and maturation of wheat plants, with no visible symptoms of injury to the plants. However, when the grain or straw from these plants was fed to experimental animals, such as rats and guinea-pigs, it produced a pronounced toxicosis characterized by retardation in growth, and death occurred in a few weeks. Wheat which was found by analysis to contain 8 to 10 p.p.m. of selenium, absorbed from the soil, produced fatal injury with, in many cases, readily detectable macroscopic changes in the liver. Fifteen p.p.m. of selenium in the soil, added as sodium selenate, produced distinct chlorosis and stunting of wheat plants.

*Toxicity of natural grains.* Franke (16) has conducted extensive feeding experiments on white rats. He found that grain from farmers whose livestock had been affected was extremely toxic to white rats. The grain had no unusual odor or taste. The pathological symptoms produced in the animals were different from those brought about by any known plant toxin, such as alkaloid, glucoside, saponin, or toxalbumin. The toxicity could not be attributed to any product of decomposition in the grains, and certainly could not be ascribed to a deficiency of proteins, carbohydrates, fats, vitamins, or minerals.

Individual rats exhibited wide variations in degree of tolerance, and affected grain from different sources showed all degrees of

potency. Lethal grain produced very severe effects, with death occurring within eight days in some cases. Sublethal grain produced growth retardation and death only after a long period of time; the least toxic grain, however, brought about only growth retardation.

Tests with the most potent of the lethal grain produced death in 321 out of 325 rats. The grain included corn, wheat, barley, and emmer. Death occurred as follows:

|                          |                    |
|--------------------------|--------------------|
| 16 per cent on or before | 25th day           |
| 39 per cent on or before | 40th day           |
| 71 per cent on or before | 60th day           |
| 92 per cent on or before | 100th day          |
| 25 per cent died between | 14th and 34th days |
| 36 per cent died between | 34th and 54th days |
| 16 per cent died between | 54th and 74th days |

Six lots of sublethal grain produced death in only six out of 52 rats by the 60th day.

*Pathology.* The pathology of rats that had been fed on poisonous grain is described in detail by Franke (16). Autopsies revealed conspicuous liver lesions. Less toxic samples of grain produced depression of growth as the only observable effect. Weaned rats placed on a diet containing a high percentage of lethal grain consumed only about one-fourth of the normal amount of food. Some of the rats lost weight rapidly; others gained weight slowly. Franke and Potter (24) proved that the pathological effect was not due merely to inanition. Rats fed on limited amounts of control grain showed no abnormalities other than stunted growth.

*Varying concentration and time.* Franke (18) made experiments on the effect of feeding toxic foodstuffs to white rats in varying amounts and for different time periods. It was found that a diet containing only 17.5 per cent of toxic grain produced definitely depressed growth rates and also caused a number of deaths. Concentrations of 35 per cent and more caused still greater depression of growth and a greater number of deaths. A study was made of the effect of diets containing toxic wheat for 30-, 20-, and 10-day periods, followed by control diet. Pathological symptoms resulted even from the 10-day period on the toxic diet, although normal growth was resumed when the rats were changed to the

control diet for 165 days. The damage to the internal organs was never repaired. The pathological changes decreased as the intake of toxic food decreased.

*Alternation of toxic and non-toxic foodstuffs.* In another set of experiments Franke (19) fed toxic and control foodstuffs alternately to rats. The intervals were 5-, 10-, and 15-day alternations of toxic and non-toxic diets. This alternate feeding gave growth and food consumption curves exhibiting rhythmic decreases and increases.

*Selenium like natural toxicant.* Franke and Potter (25) reported that the symptoms of selenium poisoning produced by feeding rats small quantities of sodium selenite in an otherwise normal diet are virtually identical with the symptoms produced by the natural plant toxicant. The evidence obtained in these experiments supports the idea that selenium is closely related to the natural toxicant. But, according to the authors, it has not yet been definitely proved that the toxicity of cereal grains is strictly proportional to their selenium content. The maximum selenium content of the naturally toxic grains from farms is about 30 p.p.m.; every grain sample known from feeding tests to be toxic gave a positive test for selenium, and non-toxic grain obtained from areas remotely separated from the affected areas gave negative tests (Franke and Painter, 22). It is possible that vanadium (Byers, 8) or molybdenum (Beath, Eppson, and Gilbert, 4) may contribute to the toxicity of some of the grains.

*Effect of toxic grains on chickens.* The low hatchability of eggs from chickens fed on toxic grains was studied by Franke and Tully (28). Monsters were produced from eggs obtained from hens on an affected farm. Approximately 75 per cent of the eggs which failed to hatch on the twenty-first day contained deformed embryos. The hatchability was only 4 to 12 per cent. Chicks from some of the eggs that hatched lived only a few hours. The down on these chicks appeared greasy and never became fluffy. When chicks were fed a ration containing 65 per cent of a toxic grain, distinctly inhibited growth resulted (Tully and Franke, 54). When they received only 25 per cent, they made practically normal growth. Egg production was delayed and reduced by the ration containing 65 per cent of affected grain. No distinct lesions of the internal organs were found from gross appearance as in the

case of rats. By injecting selenium into eggs before incubation, monsters have been produced, having beaks, eyes, and legs missing or malformed (Franke, 21).

*Anemia.* Anemia developed in white rats which were fed toxic wheat (Franke and Potter, 24, 25). The decline in hemoglobin was shown not to be due to inanition.

*Avoidance of toxic food.* Rats on a toxic diet voluntarily reduced their food intake (16, 24, 25). In experiments in which rats were given a choice between diets having various concentrations of selenium (natural or sodium selenite), the animals invariably chose the least toxic food, possibly basing their selection on taste or odor imperceptible to man (26). It is commonly believed by cattlemen that range animals are able to recognize the seleniferous vegetation (some of which possesses a telltale garlicky odor), and eat only the least toxic.

*Selenium poisoning and oxidation-reduction relations.* Schneider (48) investigated the toxic effects on rats of ingested sodium selenite incorporated in a stock diet. At a level of 70 p.p.m. it arrested the growth of 3-weeks-old albino rats and caused emaciation and death within 2 weeks. A level of 35 p.p.m. caused slight and erratic growth, death occurring in about 6 weeks. A level of 17.5 p.p.m. resulted in slightly subnormal growth and a variation in the lethal effect; some of these animals lived as long as 267 days, with no other symptoms than a slightly subnormal growth; these growth effects were shown to be due to inanition.

Adult rats withstood the poison more effectively than growing rats. On high levels, of 420 p.p.m., adult rats virtually starved to death, although autopsy revealed subacute pathological changes. The symptoms of selenium poisoning described by Schneider are apparently identical with those reported by Franke (16) for the naturally toxic grain. Schneider suggests that selenium may disturb the oxidation-reduction relations of physiology, in which sulphur normally plays an important part. He was able to show a diminished oxygen absorption by rat liver to which sodium selenite was added, but could demonstrate no diminution in the livers of rats which had previously received a selenium ration.

Further evidence that selenium is capable of inhibiting cellular respiration is seen in the work of Potter and Elvehjem (45) which showed that the rate of oxygen uptake by yeast was reduced to

one-fifth by the addition of sodium selenite when glucose, mannose, or fructose was used as substrate. The inhibition was most marked in an acid medium and fell off rapidly at pH 7.5.

*Selenium in Astragalus.* Beath, Draize, and Eppson (3) pointed out in 1932 that specimens of *Astragalus bisulcatus* (the two-grooved milk vetch) growing in some soils had an extremely offensive garlicky odor, while specimens from other soils entirely lacked this odor. It was later demonstrated (5) that the plants with the offensive odor were more toxic than those lacking it. The only variable factor contributing to this difference was the presence of selenium. This plant seems to be responsible for more losses of cattle and sheep in Wyoming than are caused by any other poisonous plant.

*Indicator plants.* Eight species of native plants, including *Astragalus bisulcatus*, were reported as indicator plants that always contain selenium when collected on soils derived from Cretaceous and Eocene shales (5). The indicator plants were richest in selenium when they occurred on the undecomposed shales. The selenium content of the plants varied from a trace to 1000 p.p.m. Range plants containing selenium were more poisonous to livestock when they grew on the Niobrara, Steele, or Pierre shales than when they grew on the other shale formations.

Since it contains an alkaloidal poison, *Astragalus bisulcatus* free from selenium is somewhat poisonous to livestock. Forced feeding trials demonstrated that 50 ounces of selenium-free *A. bisulcatus*, per hundredweight of sheep, failed to affect an animal seriously, whereas 25 ounces of the same species containing selenium produced death in a few hours.

*Selenium poisoning.* There appears to be some variation in the selenium poisoning of livestock, depending upon the species of plant ingested (5). This suggests that the various selenium-bearing plants carry the element in different chemical combinations, or that the selenium may be present with other toxic substances. But, in general, the autopsy findings of animals dying on the range of blind staggers (the term used in Wyoming to denote this type of poisoning) are in fairly good agreement with those of the experimental animals dying from the administration of small quantities of the sodium salt of selenious acid.

*Types of disease.* Draize and Beath (12) distinguish two types

of disease in livestock due to mineral poisoning—namely, alkali disease and blind staggers. Alkali disease, well known in South Dakota but rather uncommon in Wyoming, is the less acute form and is characterized by loss of hair and deformation and sloughing off of hoofs. Blind staggers, a disease of Wyoming range cattle, represents a much more acute type of poisoning, which results in death within a comparatively short period of time. There is no sloughing of hoofs or loss of hair in typical cases of blind staggers. Both diseases, however, are characterized by similar injuries to the liver. These authors believe that the two forms of disease are produced by the same agent, and that microscopic pathology hardly justifies their designation as distinct types of poisoning.

*Selenium in animal body.* Selenium, when present in food, finds its way into all the body tissues, attaining concentrations as high as 16 p.p.m. in the heart, 25 p.p.m. in the liver, and 27 p.p.m. in the blood (Dudley and Byers, 15; Byers, 9). Since selenium is present in all secretions and excretions, selenium poisoning does not seem to be permanently cumulative, even though injury to the tissues is permanent in character. According to Dudley (13), the blood of animals fed inorganic or organic compounds of selenium may contain from 7 to 27 p.p.m., thus transporting the toxic material to all parts of the body. The selenium, however, is deposited predominantly in the liver, kidney, and spleen (3 to 25 p.p.m.). Concentrations of 8 to 20 p.p.m. were also found in the hoofs. Since the bile and urine may contain as high as 5 or 6 p.p.m., excretion by hepatic and renal routes seems mainly responsible for elimination of selenium from the body. Dudley (14) found that the urine of men employed in the extraction of selenium contained from a trace to 6.9 p.p.m. A garlicky odor of the breath and other symptoms were noted.

*Fermentation.* Franke and Moxon (20) studied enzyme activity in order to develop a simple biological test for the toxicity of proteins, and to study possible effects of these toxic proteins on several important enzymes of the animal body. They found that protein from normal grain, when added to a fermenting mixture of yeast and glucose, would increase the rate of fermentation, whereas protein from an affected grain would not increase the rate of fermentation. Moxon and Franke (40), in reporting studies on the effect of selenium salts on fermentation, state that the toxicity of

sodium salts of selenite, selenide, and selenate decreases in the order named.

*Water.* Alkali water has been definitely eliminated as a possible cause of either the so-called alkali disease or blind staggers (4, 30, 34, 35). The water itself contains no appreciable amount of selenium or other toxic substance, although water with a high salt content may possibly aggravate the effect of toxic foodstuffs. In Wyoming the popular belief that the water is poisonous probably arises from the fact that selenium-bearing range plants frequently occur rather abundantly on the slopes of alkali basins (4).

*Locoism distinct.* Locoism, in Wyoming at least, does not seem to be due to selenium (4). The principal loco weeds in Wyoming are *Oxytropis saximontana* (white loco) and *O. bilocularis* (purple loco). These plants usually grow on soils derived from granites, sandstones, and volcanic ash. They sometimes grow on Cretaceous shales, and then give a positive test for selenium. Loco weeds free from selenium, when consumed in large quantities, produce the loco disease after about 50 days. The symptoms are different, however, from those of blind staggers.

#### DISTRIBUTION OF SELENIUM IN SOILS

*Areas involved.* Byers (9) has shown that soil areas of enormous extent contain selenium. It has already been demonstrated in soils and vegetation in South Dakota, Nebraska, Wyoming, Montana, Colorado, Kansas, Oklahoma, Utah, Arizona, and New Mexico. Further investigation will probably show a wide extension of the affected areas in many parts of the world. The distribution of selenium in nature is discussed by Strock (53).

*Source of selenium.* The source of selenium in soils has been shown by Byers (9) to be pyrite or similar minerals occurring in the geological formations that produce the soil. The seleniferous soils are derived, for the most part, from shales of the Cretaceous period. Such soils appear to retain enough selenium to produce toxic vegetation when the mean annual rainfall is insufficient to produce percolation through the soil profile. Seleniferous soils may be expected in arid and semiarid regions where the soils are derived from selenium-bearing strata. They probably will be found in a number of the great wheat-producing areas of the world. There are definite indications that seleniferous soils actually exist in four continents, at least.

The distribution of the selenium is not uniform, either in the surface soil or in the soil profile. There are indications of a zone of selenium concentration in the soil profile, either within or close to a zone of sulphate accumulation. Sandy soils will probably be found to contain selenium in only small quantities, because of low original content and more complete leaching than from heavy clay soils.

*Geology.* Beath *et al.* (4) have shown that selenium is distributed in Wyoming throughout shales of the Cretaceous and Eocene periods, and that its concentration is comparatively high in Steele, Pierre, and Niobrara formations. These writers give an excellent description of the geology of Wyoming shales, based on the work of Dr. S. H. Knight, state geologist of Wyoming. Beath (2) has also found selenium in red soil derived from Chugwater, which is of the Permian or Triassic (?) age.

*Soil enrichment.* Although Niobrara shale *in situ* usually carries only 2 to 4 p.p.m. of selenium, soils derived from this shale may show an enrichment which results apparently from drainage of soluble residues of selenium-bearing plants (4). Soil at a depth of 36 inches contained 22.5 p.p.m. of selenium, of which approximately one-third was water-soluble.

*Fertilizers and insecticides.* Selenium may occur in soils to which it has been added as an impurity in superphosphate and ammonium sulphate (46, 49, 50, 51) and in soils contaminated with fungicides and insecticides containing it (29, 38, 41, 52).

#### ABSORPTION AND ACCUMULATION OF SELENIUM BY PLANTS

*Influential conditions.* The absorption and accumulation of selenium by plants growing on seleniferous soils are dependent upon two groups of conditions. I. Soil conditions: (1) Concentration of selenium in the soil; (2) nature of the selenium compounds, especially their solubility in water; (3) other soil components, especially available sulphur; and (4) seasonal variation in rainfall. II. Plant conditions: (1) Kind of plant; (2) part of the plant examined; and (3) stage of growth.

*Plant symptoms.* Studies by Hurd-Karrer (32) showed that the characteristic symptom of selenium injury in wheat plants is a snow-white chlorosis. This was produced by sublethal concentrations of sodium selenate (15 to about 30 p.p.m. selenium in Key-



port clay loam). When the selenate was added to pots containing older plants, the white chlorosis appeared only on leaves emerging subsequent to the addition of selenium, those already formed merely turning yellow. A progressive diminution of chlorosis on successive leaves was observed as the plants became older.

*Absence of symptoms in the field.* It is worthy of emphasis in this connection that symptoms of selenium injury of plants have never been reported from observations in the field. One would never suspect from the appearance of wheat, cabbages, etc., that they have accumulated dangerous quantities of selenium.

*Replacement of sulphur.* Cameron (10) suggested that selenium might produce its injury to plants by replacing sulphur in some organic compound. Levine (36) also considered that selenium might replace sulphur in plant metabolism. Brenner (7) thought that it might replace sulphur in the metabolism of sulphur bacteria.

*Selenium in protein.* Selenium absorbed by wheat is associated with the protein of the grain, possibly replacing sulphur (Nelson, Hurd-Karrer, and Robinson, 41). Franke (17) showed that the toxicant is carried in the protein fraction of toxic wheat and toxic corn. This was determined by feeding experiments. Franke and Painter (22) found selenium in the proteins of toxic corn, wheat, and barley, and in the proteins of animals that had been fed toxic foodstuffs. The selenium was in organic combination in the protein (probably replacing sulphur) and was in solution after the protein had been hydrolyzed. Painter and Franke (43) developed a method of removing selenium from toxic protein hydrolysates, through the use of butyl-alcohol extraction. A procedure with mercuric chloride was devised whereby all the selenium compounds were precipitated, mercury salts being superior to other amino-acid precipitants. The molar selenium-sulphur ratio, according to Painter and Franke (42), in a toxic protein investigated was 1:148. The effect of chemical treatment of the proteins from toxic wheat was studied by animal feeding (Franke and Painter, 23). The hydrolysates of toxic proteins were toxic to rats. But mercuric chloride precipitation of toxic hydrolysates removed the selenium sufficiently to render the filtrate innocuous when fed to rats.

*Sulphur antagonism.* Hurd-Karrer (31, 32) made an important discovery when she found that the toxicity of sodium selenate

is determined by the amount of sulphur available to the plants. In water cultures, selenium concentrations as low as 0.1 p.p.m. produced distinct injury in wheat plants after a few weeks with nutrient solutions containing no sulphate, whereas a concentration of 18 p.p.m. was required for this degree of injury in solutions containing 192 p.p.m. sulphur. There was no visible injury to the plants where the proportion of selenium to sulphur was 1:12 or less, the point of minimum detectable injury lying between 1:9 and 1:11. When the ratio was 1:8 or greater, the plants were chlorotic and stunted; and when the ratio was as high as 1:2, growth was almost completely inhibited. In soil cultures, selenium injury could be inhibited by the addition of excess sulphur, as sulphates or as elemental sulphur. The amount required varied with the toxicity of the selenate in the particular soil.

Chemical analyses showed that the injured plants contained large amounts of selenium in their tissues, whereas plants grown with these same concentrations of selenium, but with sufficient sulphates or sulphur to reduce or prevent the injury, contained much less (32). The absorption of selenium appeared to be determined not by its absolute concentration in the soil or nutrient solution but by its relative concentration with reference to the available sulphur. Applications of gypsum or elemental sulphur reduced the absorption by wheat of the naturally occurring selenium in the soil, as well as of that added as sodium selenate (33). This selenium-sulphur relationship seems to constitute a hitherto unreported instance of antagonism.

It has not been proved, however, by field tests that application of sulphur to seleniferous cultivated land offers a practical means of preventing grains from accumulating toxic concentrations of selenium.

*Conditions governing absorption.* Further greenhouse experiments by Hurd-Karrer (33) illustrated the effects of various conditions on the absorption and resulting toxicity to wheat of selenium added as sodium selenate to the soil. These included soil type, percentage of sand, method of adding selenium, the form of selenium added, and growth of previous crops. Sodium selenate was more easily absorbed by wheat from Pierre clay (a naturally seleniferous soil) than from Keyport clay loam. The greatest accumulation was 1350 p.p.m., found in plants fatally injured by

20 p.p.m. of selenium as sodium selenate added to Pierre clay. The addition of quartz sand to Keyport clay loam increased the toxicity of sodium selenate in proportion to the percentage of sand in the mixture. Sodium selenate was not easily leached from Keyport clay loam, being at least partially retained in the upper layers when a solution containing it was poured on the surface; solutions originally toxic were found to be non-toxic after being filtered through this soil. The presence of sand increased penetrability. Elemental selenium was apparently unavailable and non-toxic to wheat plants, at least in quantities up to 200 p.p.m. in Keyport clay loam. The selenium in sodium selenate proved to be more available and more toxic to wheat than that in sodium selenite. The growth of successive crops of wheat changed sodium selenate to a less toxic form or reduced it to a subtoxic concentration.

*Field surveys.* The extensive field survey of Byers (9) showed that wherever selenium was present in the soil, all kinds of plants absorbed some of this element, in amounts varying from mere traces to thousands of parts per million. Sufficient data are not yet available for an exact statement, but in general it appears that any soil containing more than 0.5 p.p.m. of selenium or any vegetation containing over 5 p.p.m. is potentially dangerous. The possibility of injury to the consumer depends of course upon the fraction of seleniferous food in the total diet.

All soils that contain selenium do not produce toxic plant materials, according to Byers (9). Certain areas bear little or no toxic vegetation even where considerable quantities of selenium occur in the soil. These unexplained variations suggest an important problem for further research. In some cases a high sulphur-selenium ratio of the soil seemed clearly to limit the accumulation of selenium in the plants.

*Differences in the ability of plants to accumulate selenium.* Analyses presented by Byers (9) demonstrate striking differences in the ability of various plants to accumulate selenium. Certain plant species, such as native prairie grasses, show relatively slight ability to take up selenium. Other species, including some legumes and composites, show a marked tendency toward ready absorption. The cultivated cereals seem to be intermediate in character. Some examples may be cited: Western wheat grass growing on various soils accumulated from 1 to 60 p.p.m. of selenium. *Astragalus*

*bisulcatus* (a wild legume) accumulated from 200 to 4300 p.p.m. A striking contrast is obtained when we compare two closely related species of *Astragalus*. Thus *Astragalus bisulcatus* on soil containing 2.1 p.p.m. stored 1250 p.p.m., while *A. missouriensis* on the same soil accumulated only 3.1 p.p.m.

A conspicuous feature of the data is the general indefiniteness of correlation between the selenium content of the soil and the selenium content of a given kind of plant (9). For example, from one soil containing 2.5 p.p.m. of selenium wheat accumulated 45 p.p.m.; from another soil containing 3.0 p.p.m. wheat took up only 0.5 p.p.m. From six soils with 1.5 p.p.m., wheat absorbed 1, 1, 2, 7, 7, and 10 p.p.m. From a soil containing 6 p.p.m. Western wheat grass absorbed 60 p.p.m., whereas from a soil with 20 p.p.m. this plant took up only 12 p.p.m. For Western wheat grass the ratio of selenium in the plant to that in the soil in twenty-two cases varied from 0.3 to 14.0, with no indication of a mean. Chemical analysis of the soil would therefore not enable one to predict the amount of selenium that wheat, Western wheat grass, alfalfa, *Astragalus bisulcatus*, etc., would absorb. This low degree of correlation may result from differences in availability of the selenium, in the effects of sulphur or other soil constituents, in distribution not represented by the sampling, in age of the plants, or in other unknown conditions. It suggests very interesting problems for further research.

Beath, Eppson, and Gilbert (4) divide species of native plants in Wyoming into three groups, according to their ability to accumulate selenium: (1) Species of the first group invariably contain selenium when collected on Cretaceous and Eocene shales. This group includes *Astragalus bisulcatus* and five other species of *Astragalus*, *Mentzelia decapetala*, *Oenopsis condensata*, two species of *Xylorhiza*, and two species of *Stanleya*. The highest recorded content is for a sample of *Astragalus bisulcatus* with 8840 p.p.m. (2) Species of the second group of native plants vary in their selenium absorption, with tests in some cases negative and in others positive. These plants include five species of *Atriplex*, native grasses of various kinds, and twelve other species. (3) Species of the third group, which include thus far 33 kinds of native plants, contain no selenium even when growing on seleniferous soils.

It is remarkable that four species of *Astragalus* collected on the

same shales and in close proximity to the selenium-bearing species have consistently been found to be free from selenium or to contain mere traces (4). No clue has yet been found to these striking differences in the ability of closely related species to absorb and accumulate selenium. This constitutes one of the most interesting of the unsolved problems.

Hurd-Karrer (33) grew seventeen different crop plants in Keyport clay loam with 5 p.p.m. selenium added as sodium selenate. Broccoli and mustard, of the Cruciferae, absorbed more selenium than any others—i.e., 1180 and 1240 p.p.m., respectively. She suggested that the tendency of a crop to absorb selenium depends on its tendency to absorb sulphur.

*Selenium converters.* Beath and his associates (4, 5, 6) have advanced a very interesting hypothesis regarding the part played by certain native plants. Their idea is that certain native selenium-bearing plants, such as *Astragalus bisulcatus*, absorb selenium from virgin shale soils, convert it into water-soluble forms, and return it to the soil in forms available for absorption by other types of plants, including farm crops. These native plants are therefore regarded as selenium converters and soil contaminators.

The toxicants of the selenium-bearing range plants could be extracted freely with water. Through the decay of foliage, seeds, and roots of these plants a considerable amount of this element may be supposed to go back to the soil in forms readily available to any type of plant. The chemical nature of the water-soluble selenium compounds has not been described thus far, nor is it known what substances are responsible for the offensive odor of *Astragalus bisulcatus* and *Oonopsis condensata*. Water extracts of green *A. bisulcatus* mixed with crude undecomposed Niobrara shale in experimental plots imparted recognizable amounts of selenium to barley grown on the plots. Barley grown on the same shale composite without the addition of the vetch extract did not absorb selenium. Native grasses growing in close proximity to selenium-bearing range plants were found to be poisonous to guinea pigs. A number of other native plants were found to contain selenium when influenced by the plants that yielded soluble selenium compounds, but the same species of plants were selenium-free when grown on uncontaminated shales.

*Selenium absorption by crop plants.* The absorption of appre-

ciable amounts of selenium by farm crops, such as cereals and forages, is regarded by Beath and his co-workers (4) as dependent upon previous enrichment of the soil by converter plants. Tests showed that wheat and oats absorbed dangerous quantities of selenium if sown on soil prepared by ploughing under a heavy stand of native selenium-bearing plants. Whenever toxic corn, oats, wheat, and cultivated forage crops have been found in Wyoming, it has been possible to obtain evidence that the virgin soil recently supported dense growth of selenium converters, or had been enriched by drainage from these plants (4). Ordinary crops absorb little or no selenium in most of the farming areas of Wyoming, even though the soils themselves show the presence of selenium. This indicates that selenium is rarely, if ever, present in these areas in a form available to ordinary cultivated plants.

*Small absorption from raw shales.* Farm crops were grown upon raw Cretaceous shales taken from naturally occurring deposits so selected as to exclude any possibility of their having previously carried a growth of vegetation (4). It was found that these plants contained only very small quantities of selenium. The highest concentration was 2 p.p.m., in wheat heads. Some other crop plants, such as alfalfa, cabbage, sugar beets, and hairy vetch, contained no selenium. Peas contained 1.7 p.p.m.; crested wheat grass, 0.3; oat heads, 0.7; corn, 0.6; beans, 0.4; carrots, 0.3; parsnips, 0.2; rutabagas, 0.5; barley heads, 0.1; potatoes, 0.1; and sunflower seeds, 0.6.

It seems unlikely to Beath and his associates (4) that cultivated crop plants are rendered poisonous to livestock by the small quantities of available selenium present in soils derived from raw shales. Unfortunately, precise information on what constitutes a harmful amount of selenium in such crops is not yet available. It may be said, however, that barley hay containing 6 p.p.m. of selenium was fed to cattle for several months without producing symptoms of injury, and that salt bush containing 19 p.p.m. of selenium was fed to cattle and hogs for three months without noticeable ill effects. Attempts to produce alkali disease in cattle, sheep, and hogs by feeding wheat and barley containing only a few parts per million of selenium were unsuccessful.

Although Byers (8) reported that wheat accumulated 25 p.p.m. of selenium when grown on Pierre loam containing 2 p.p.m., Beath

and his associates (4) found that wheat accumulated only 2 p.p.m. of selenium when growing on raw Steele shale containing 2.4 p.p.m. To enable wheat plants to accumulate 25 p.p.m. of selenium, raw shale had to be sufficiently enriched with concretions to raise the selenium content to 65 p.p.m.

*Action of soil contaminators.* A striking contrast to the preceding results is seen in an experiment in which wheat was grown on a shale soil that had previously borne a dense growth of the selenium converters *Astragalus bisulcatus* and *A. pectinatus* (4). Although the soil contained only 1.1 p.p.m. of selenium, this appeared to be in a form easily available to wheat, for the plants accumulated 45 p.p.m. In another experiment a crop of barley was grown on a soil that had carried a heavy stand of *Astragalus*. The straw and grain, when fed to cattle, produced typical symptoms of alkali disease after six weeks' time. Chickens that ate the grain lost their feathers and many of them died.

*Alfalfa.* Alfalfa has not been reported as producing selenium poisoning of livestock in Wyoming (4). Chemical analyses of alfalfa from selenium-bearing raw shales have been negative or have shown only a few parts per million of selenium. Even when alfalfa was grown on a plot that previously bore a dense stand of *Astragalus pectinatus* and *A. bisulcatus*, the crop gave negative tests for selenium, both by chemical analyses and feeding trials with rabbits for two months.

*Variations.* Although, in general, the abundant forage plants on the ranges in Wyoming are free from dangerous quantities of selenium, there are some interesting and as yet unexplained variations. For example, *Eurotia lanata*, one of the most desirable forages on Wyoming ranges, was found to contain 23 p.p.m. of selenium on one Niobrara outcrop, whereas it was entirely free from selenium on a similar soil. Certain rather limited grazing areas in Wyoming are so dangerous that they have come to be known as poison areas. They usually bear dense growths of woody aster, *Oenopsis*, and species of *Astragalus*, and there are indications that even some of the native grasses may contain sufficient selenium to be injurious.

*Is the toxicity of grains always dependent upon action of selenium converters?* Although Beath and associates (4) have presented abundant evidence that converter plants are able to make

selenium available to crops, it has not yet been proved that the toxicity of grains in seleniferous areas outside of Wyoming is dependent upon the action of converter plants. Some of the soils that now produce toxic grain have been in cultivation for the last twenty-five or fifty years, and certainly have not recently supported dense stands of *Astragalus bisulcatus* or other selenium-accumulating wild plants. Further research will be needed to show how important a rôle the converter plants may have played in the cereal-growing regions where alkali disease has been prevalent for so many years.

#### CONTROL AND PREVENTION

Much more research work will need to be done before adequate methods of control and prevention of selenium poisoning can be applied. The following control measures have been suggested by Franke *et al.* (27): Toxic grain, hay, or grass should not be used for feed or pasturage. Affected animals should be transferred to unaffected areas. Areas definitely known to produce toxic grain and forage should be left uncultivated, and suspected areas should be studied to determine definitely whether or not they produce toxic vegetation.

Byers (9) has added the following recommendations: It would seem wise to withdraw toxic arable land from cereal production. The exact extent of affected areas should be determined, and remedial measures applicable to field conditions should be sought. Irrigation areas should be examined and controlled. Stock raising in seleniferous areas seems practicable if the ranges are not overgrazed. Analyses have shown that as a rule the native forage crops, such as buffalo grass, grama grass, bluestem, and needlegrass, contain relatively small quantities of selenium. Observations have indicated that animals learn to avoid seleniferous vegetation; even pigs avoid foods containing small quantities of inorganic selenium compounds (cf. 26). A study of toxic limits, tolerance limits, diagnostic symptoms, and remedial measures should be undertaken. Tolerance limits should be set up as soon as possible; but the emergency is not serious enough to warrant hasty measures. Above all, the proper safe-guarding of public health within the affected areas should be studied.

Beath *et al.* (4) suggest the following corrective measures as



applicable to Wyoming: Grazing of livestock should be avoided on areas bearing dense stands of seleniferous native range plants, and over-grazing should be prevented. Destruction of dangerous plants on desirable ranges by grubbing may be practicable in some areas; these plants are poisonous themselves, and they convert selenium into water-soluble forms that may be absorbed by desirable range plants. Revegetating with forages which are not selenium accumulators may be feasible. Ranchers should be advised regarding the kind of livestock best adapted to particular ranges. Cultivated fields that produce crops and forages toxic to livestock should be left uncropped until more information is available concerning types of feed and forage plants suitable for use on such areas, and until more is known about the amount of seleniferous grain and hay which the animals may tolerate. Although addition of sulphur to cultivated land offers a possible means of preventing grains and forages from absorbing toxic quantities of selenium, the shales and the soils derived from them are normally sulphurized, and successful treatment by this method may not be practicable. In the large grazing areas soil detoxification obviously would be too expensive. Drainage may serve to reduce the selenium content of irrigated soils.

#### POSSIBILITY OF HUMAN INJURY

*Selenium in foods.* Byers (9) gives a table that shows potentially dangerous selenium concentrations in some common foods from a naturally seleniferous area. The highest selenium contents, in p.p.m., are as follows: Cabbage, 100; turnip leaves, 25; eggs, 10; lettuce, 7; wheat, 5; rye, 5; corn, 3; string beans, 2; and milk, 1.5. Franke and Painter (23) report 31 p.p.m. in whole wheat and 120 p.p.m. in the protein of the grain. Using soil fortified by the addition of 5 p.p.m. of selenium, as sodium selenate, Hurd-Karrer (33) obtained much higher values, which are as follows, in p.p.m.: Mustard, 1240; broccoli, 1180; spinach, 315; young wheat, 470; young barley, 450; and young corn, 275.

*Affected areas.* In the more seriously affected areas human injury might be extensive if the inhabitants provided a considerable fraction of their diet from their own produce (9). Small mills, however, no longer exist, and the farmer receives his flour from Minneapolis or other distant milling centers. Relatively few vegetables are raised locally.

*Country as a whole.* The danger to public health outside the selenium area seems relatively slight. Even when toxic wheat is marketed, its dilution with non-toxic wheat and the small fraction which bread constitutes of the normal diet would tend to render it practically innocuous (9). The problem deserves careful study. Methods need to be devised for diagnosis of incipient selenium poisoning (Dudley and Byers, 15). Tolerance limits, toxic limits, and remedial measures should receive thorough investigation. The research required on human phases of the problem seems barely to have been touched. The problem is not confined to the United States; for, according to Wilcox (55), selenium has already been found in wheat from Canada, Mexico, Argentina, Australia, New Zealand, South Africa, Algeria, Morocco, Spain, and Bulgaria.

*Insecticides.* Nelson, Hurd-Karrer, and Robinson (41) have warned against the use of selenium compounds as insecticides, since there is considerable danger from even minute quantities of selenium in soils on which food products are grown. Even spray residues ordinarily considered innocuous may be made available to the plant and be accumulated in toxic amounts.

#### TOXICITY OF OTHER MINERAL ELEMENTS

*Molybdenum.* Most plants growing on Cretaceous shales have been found by Beath, Eppson, and Gilbert (4) to absorb molybdenum in varying amounts. Some plant species, particularly *Oonopsis condensata* and *Xylorhiza parrii*, contain fairly large quantities. One sample of *Oonopsis* showed 317 p.p.m. of molybdenum. When barley was grown on soil to which sodium molybdate had been added, the hay contained 89 p.p.m. of molybdenum. The hay was fed to livestock, and produced erosion of long bones and some other symptoms similar to those brought about by selenium. Morrison shale, even when uncontaminated through the activity of *Astragalus bisulcatus*, etc., seems to produce poisonous cereals, root crops, and forages. The toxicity of these crop plants seems not to be dependent on their selenium content. Possibly molybdenum is responsible.

*Tellurium.* Tellurium is found in only a few native plants in Wyoming (4). A cactus showed 25 p.p.m. and salt bush 2 p.p.m. No tellurium has been found in crop plants growing on farms, but wheat seedlings grown in soil containing potassium tellurite accu-

mulated 507 p.p.m. It is to be expected that plants containing tellurium will be much less toxic than those containing selenium.

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VOL. II

AUGUST, 1936

No. 8

## PALAEOBOTANY AND THE ORIGIN OF THE ANGIOSPERMS

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The origin of flowering plants is still one of the greatest problems of evolution and has a direct bearing on the taxonomy of the group. The questions at issue concern not only the identification of the more primitive families but the morphological nature and origin of the flower, and they may lead us to ask how and why the classical concepts of floral morphology have originated. The problem has been hitherto studied almost entirely by the comparison of forms now living, and though a vast amount has been written no certain or agreed conclusions have been reached. Is it possible that palaeobotany can make a real contribution to the discussion? We can never hope to find the remains of complete lineages of ancient plants; the conditions under which their preservation could have occurred were too rare. But we do find examples of vegetation taken in a random way over a period of 300–400 million years, and these ought to give us some general idea of the evolutionary trends in plant structure over the period. Our samples are mainly the plants of low-lying areas near deltas and estuaries, and their probable relation to their contemporary forms should be assessed with this in mind.

At the outset we are faced with the question of the characters which distinguish angiosperms from other plants. Assuming that gradual morphological change has taken place during the evolution of the group, how are we to distinguish a primitive flowering plant if we find one? The possession of a closed ovary is not enough, for in the past plants which were far removed from the main assemblage of modern flowering plants, existed (68) with this peculiarity. Leaf form certainly underwent great change. Stem structure is not decisive. The most reliable single character is the

possession of flowers. But we must then decide what we mean by a flower. Almost all botanists are agreed that the flower is "a specialized strobilus" (or a bud) "in which the lower sporophylls have become sterilized to form sepals and petals and the upper have changed into stamens and carpels" (66). The axis of the strobilus is thought to have shortened to form the receptacle in hypogynous flowers of the primitive type, while in the more specialized types it has become depressed into a cup containing the carpels. Few modern writers specify exactly what they mean by a sporophyll, but the term implies a foliar structure or a modified leaf. Inquiry into the origin and development of the concept shows that it originated with Goethe in pre-evolutionary days as a subjective generalization (71) for which objective evidence could be neither expected nor required. This generalization was taken up by Darwin and used as an argument for evolution, since when it has been regarded as too axiomatic to need demonstration. Consequently, what was a mental picture has assumed a historical significance, and no serious attempt has been made to verify or disprove it. Now if this classical view of floral morphology, with its implications as to the course of floral evolution, is true, it should receive support from historical evidence of the rocks. We have large samples of undoubted flowering plants preserved at frequent intervals over some 90 millions of years, and these ought to provide evidence of the progress from hypogynous to peri- and epigynous flowers and of a progression from sporophylls of a more leaf-like to less leaf-like types. Is there such evidence?

#### FOSSIL PLANTS AS TESTS OF CURRENT THEORIES

In endeavoring to trace the history of our flowering plants backwards we may notice that our samples from Tertiary and Upper Cretaceous rocks (see Appendix) are numerous (100-400 species) and come from different parts of the world; the majority, however, can be assigned to living families. A number of Pliocene and more recent fossils can be identified with living species, but while the generic identification of Miocene angiosperms is clear, many of the species are no longer referable to living forms. Of the Eocene species identified by fruits and seeds, only some 25 per cent of the floras can be referred to living genera, but in their leaves, seeds and fruits most of the remainder are so close to living genera that their



general affinities cannot be questioned. Thus, while there has been morphological change during the last 60 million years or more, it seems to have been very slow in the many plants which occur in our records.

Before we can attempt to assess the direction of the changes we must be sure of our identifications. Up to the present, the angiosperms in Tertiary and Cretaceous floras have been identified mainly from leaf impressions in fine grained rock. Hooker long ago (34) cast doubts upon the specific identification of fossil plants, and many authors have since felt that leaf remains had too often been identified from evidence which no botanist would accept in work on modern plants. But when the leaves are found as really well preserved or mummified specimens, and comparison is made with a wide range of herbarium material (17), significant determinations may be reached. Recent study of cuticular characters has added many additional features for comparison and has led to the recognition of several genera not previously known from Tertiary rocks (4, 5, 6, 7, 31, 32, 65). The value of cuticle characters has been questioned (50) but a critical survey of the whole field by Edwards (20) shows their undoubted importance. The collection and identification of fruits and seeds which are abundant in certain fresh-water deposits confirms much of the evidence from leaves and adds information which is of the greatest significance.

In this field of study the Pliocene beds have provided rich seed floras (51).<sup>1</sup> Miocene seeds and fruits are known from several European localities (82, 39). Many forms are known from the Oligocene (39, 52), while most important work has been done on the Eocene floras (16, 53).<sup>1</sup> The extraction and identification of seeds from fresh-water rocks in North America has been commenced (21), but it seems certain that very much remains to be done.

A distinct and new line of progress has come with the collection and study of pollen from shales and other rocks where it is often abundant. Wodehouse (80, 81) has identified more than 100 species among a large number of distinct forms of pollen grains from the Eocene Green River formation, and a commencement of this very promising work has been made in Europe. The number of well preserved remains of flowers is small but not inconsiderable

<sup>1</sup> The references given comprise only a small selection from the literature.



specimens, like the well-known flowers in amber (17A), are of importance as showing how little has been the change in floral structure over the many millions of years since Oligocene times.

One of the oldest sources of evidence about the Tertiary dicotyledons is derived from the study of petrified woods, of which a large variety has been found in different strata and areas. The majority were studied and named a long time ago when our knowledge of wood anatomy was far from complete; their suggested affinities, consequently, are very doubtful. Even at the present time authorities are uncertain of the extent to which genera and even families can be recognized by the structure of their secondary wood, but there can be little doubt that the recent activity in this branch of study will be of great value to palaeobotany. A valuable prelude to a reconsideration of the whole field has been the production of a catalogue of all the described species (19), and critical studies of certain groups have been undertaken (2, 3). All we can now say is that between 40 and 50 families appear to be represented by the Tertiary woods so far described.

Combining the evidence from all the different lines of work we can now be quite sure of the general succession of plant forms on the shores of the estuaries in North Temperate regions throughout the Tertiary period, in terms of the existing flora of the world, and that a comparatively small percentage of the forms discovered are totally unlike any living species. Some of our recent progress has been summarized by Hirmer (30) who gives lists of the species found. With these before us it should be possible to gauge the trend of floral change.

A comparison of the modern flowering plants with those of the Lower Eocene, which must be about 60 million years old, ought to provide significant results. From the London Clay, Reid and Chandler (53) have recognized about 314 species of fairly well preserved fruits and seeds, 59 could not yet be referred to a family and 234 were given specific names. The genera distinguished numbered about 100, belonging to 43 families, and 61 genera could be closely compared with 53 modern genera. If the floral structure of these modern genera is analyzed, we find the forms with perigynous flowers or with stamens inserted on a disc vastly outnumber the hypogynous forms with a convex torus; in fact, the genus *Magnolia*, represented by 10 species, is the only one whose flowers are

of the supposed primitive type. The best represented families are the Lauraceae, Icacinaceae, Euphorbiaceae and Anonaceae, while 7 sympetalous families are represented. Furthermore, the Nymphaeaceae is represented only by a form allied to *Barclaya*, the one member of the existing family which has perigynous or epipetalous stamens.

Following back the record of flowering plants to the Upper Cretaceous rocks, we find large floras in different parts of the Northern Hemisphere in which conifers played an important part. Unfortunately we can no longer place such implicit reliance on our identifications because they are based mainly on leaf impressions, but the general support recently given to the identifications of Tertiary leaves as indicated above, and the occasional discovery of recognizable fruits and seeds, suggests that the published lists of Cretaceous plants may give us a fairly accurate picture of the flora. The most important recent works have come from Berry (12-15) and Hollick (33) in North America. Seward has reinvestigated the flora from Greenland (61) while work has been done on a Central European flora of Cenomanian age from Czechoslovakia (74). From these papers it is clear that palms and certain other monocotyledons grew with a considerable variety of dicotyledons. The larger American collections suggest the presence of some 30 families in 20 or more orders, and, while a considerable number of forms appear to belong to extinct genera, most of them are thought to be near enough to modern genera to allow their attribution to families. If this has been done correctly, sympetalous forms are represented by about 10 per cent of the dicotyledons in each flora. There still seems to be no marked preponderance of genera with any single type of flower but the Polycarpicae of Wettstein are especially well represented. The Lauraceae are noticeably abundant while Magnoliaceae and Menispermaceae are also prominent. Sapindales, Rhamnales and Umbellales are thought to be well represented. In each flora many leaves are attributed to *Ficus*, and at several localities the remains of fruits have been discovered, while leaves with fruits referable to *Artocarpus* have also been recorded. Two families now especially characteristic of the Southern Hemisphere are frequent in Europe and North America, the Myrtaceae, on the evidence of leaves and of fruits identified as *Eucalyptus* (73), and the Proteaceae on the evidence of leaves, and the remains

of inflorescences (75). An independent line of evidence comes from the discovery of petrified wood. Specimens from North America, Europe, Egypt and Japan have been compared with the groups Caesalpinoideae, *Euonymus*, *Fagus*, *Juglans*, *Laurus*, Piperaceae, *Nothofagus*, *Phyllanthus*, *Populus*, *Rhus*, Celastraceae, *Sabia*, Saururaceae, Sterculiaceae (19). While the value of these comparisons is doubtful they should at least indicate that the flora was of a varied character. Our direct knowledge of flowers from this period is small but cannot be neglected. Stopes and Fuji (64) described a petrified form from Japan which had a trilocular ovary not more than 3 mm. high and slightly inferior, a perianth or disc being fused to the lower part of the ovary. Flowers or fruits of twelve different forms have been recently described from the Cenomanian of Czechoslovakia (74). Some of these have been compared with fruits or flowers of *Rhizophora* and *Celastrus*. The types *Rutaecarpus* and *Ceratocarpus* had syncarpous ovaries. *Triplicarpus* is figured as a whorl of fruits suggesting affinities with the Anonaceae, while fossils compared with flowers of *Myrica* and *Sparganium* have also been figured.

The records of the Lower Cretaceous rocks are interesting for the lower strata, such as the Patuxent series of America and the Wealden of Europe, contain only typical Mesozoic floras. There are no indubitable dicotyledonous leaves though three forms, called *Proteaephyllum*, *Ficophyllum* and *Rogersia*, have reticulate venation, possibly suggesting angiosperm affinities (11). The uppermost strata, however, contain an appreciable percentage of forms referable to the flowering plants and these again show considerable variety and seem to resemble a wide range of families. Some years ago, Saporta (56) identified about twenty genera of dicotyledons from the Albian rocks of Portugal on leaf impressions comparable with *Cissus*, *Magnolia*, *Nelumbium*, *Eucalyptus*, *Sassafras*, *Laurus*, *Myrica*, *Salix* and other forms. From the Patapsco beds of Maryland, Berry (11) described three fossils regarded as monocotyledonous together with a variety of dicotyledonous leaves. The names given to these types indicate their general aspect and these are *Populus*, *Populophyllum*, *Nelumbites*, *Menispermities*, *Sapindopsis*, *Celastrophyllosum*, *Cissites*, *Sassafras*, *Araliaephyllum*. In addition, there were five genera of unknown affinities, possibly dicotyledonous. It is impossible to say whether the implied affini-

ties of these leaves can be substantiated, but there seems to be no reason why they should not be generally correct. In any event, we see that from the time when the angiosperms first became dominant in the floras of the marshes or estuaries they were represented by a wide range of types, clearly belonging to several divergent families. This conclusion is still more firmly established by our knowledge of Lower Cretaceous petrified woods. From beds of Senonian age come types named *Carpinoxylon*, *Cornoxyton*, *Fegonium*, *Juglandinum*, *Laurinum*, *Plataninum*, *Salicinum* and *Taenioxyton*, from the Cenomanian were derived *Hamamelidoxylon* and *Salicinum*, from the Albian a *Laurinum* and from the Aptian, *Aptiana*, *Cantia*, *Woburnia* (*Dipterocarpoxyton*), *Hythia* and *Sabulia*. The Aptian woods from the Lower Greensand of England are among the earliest known dicotyledons of Europe and it is interesting to note that they were described by Stopes (62) as highly specialized types showing "little evidence of any primitive features." On the other hand, a number of these Lower Cretaceous forms show scalariform perforations, and several may be described as of a generalized type which appears today in such families as the Myricaceae, Theaceae, Myrtaceae, Tiliaceae, Ericaceae, Symplocaceae, Rubiaceae and Caprifoliaceae (2). It should be noticed that there is independent evidence from leaves or fruits suggesting the presence of several of these families in the Cretaceous or Lower Tertiary periods. In far eastern Siberia a few angiosperm-like leaves, named *Aralia*, *Proteaephyllum* and *Pandanophyllum*, appear in Aptian beds associated with Wealden species (45).

The study of Cretaceous floras seems, then, to indicate the sudden rise to dominance of angiosperms during this period, and has given rise to the view that about this time the flowering plants evolved with extreme rapidity from some unknown but widely different ancestors; but the facts do not justify this conclusion. All we can say is that during this period the angiosperms replaced many of the older gymnosperms in the floras of the estuarine and marsh lands. They must have long existed on dry ground and their spread may have been accelerated by the appearance of birds, or by some other biological factor. There is also the possibility that the distinctive dicotyledonous type of leaf was steadily evolving at this time from some other types which may have been found in older rocks but have not suggested angiospermous affinities. The far

greater antiquity of the group is proved by a few rare finds from Jurassic and Triassic rocks. Seward (58) described a leaf from the Stonesfield Slate (Jurassic) of England. A leaf (55) from the Solenhofen beds in Germany has an outline more like that of a compound dicotyledonous form than anything else. A piece of silicified wood (42) from the Brown Jura of Germany seems to have vessels with the ring-pore arrangement and may be a dicotyledonous type. While a silicified wood from India, probably but not certainly Jurassic, has no vessels but is comparable with the homoxylous dicotyledons in structure (54). This last example might, of course, belong to some gymnosperm at present unknown or to one of the Bennettiales. Its affinities and signification have given rise to some discussion (24, 79). Beyond the Jurassic the rocks of the Trias have furnished two interesting specimens. *Furcula* is a narrow leaf with a forked lamina, a reticulate venation and stomata of the angiospermous type, which was found by Professor Harris in Greenland (27). From rocks in South Africa, thought to be of a slightly older date, comes a structure not yet described in print and which, though not well preserved, can be compared only with an inflorescence bearing flowers. These have a whorled perianth of five (or possibly six) segments and a short or disc-like receptacle. Neither stamens nor carpels can be made out, but even in the absence of conclusive evidence the mere existence of structures of this type as early as the Middle Trias is most interesting and raises fresh doubts about the validity of the classical theory of the flower.

Professor Wieland has suggested dicotyledonous affinities for the winged seed or fruit called *Fraxinopsis* (78) found in the Rhaetic of Argentina, and later in Japan and Australia. A mere external similarity to *Fraxinus* does not, however, enable this suggestion to be received with confidence, especially in the light of recent discoveries of new plant groups in the Mesozoic. The Bennettiales and Caytoniales were of some importance in the Mesozoic floras but when we are working backwards it is very difficult to connect them directly with flowering plants, though they may well be distantly related through common ancestors. The Caytoniales show little or no approach towards flower formation though they were angiospermous in the Jurassic, while the Bennettiales, which had flower-like aggregations of their fertile organs, differ in most

of their structural details from the flowering plants and were undoubted gymnosperms.

The attempt to trace the history of the flowering plants backwards makes it clear that their ancestry must go far back to the early Mesozoic or Palaeozoic period. The historical sequence of forms shows that evolutionary change has been very slow, at least in many groups. None of the evolutionary schemes hitherto proposed for the dicotyledons seems to agree with the historical evidence which, moreover, gives no support to the current interpretation of floral morphology. On the other hand, we find that plants with small carpels, with whorled stamens and with the receptacle forming a disc-like or perigynous structure predominated among the earliest known floras. If any backward convergence of type is discernible, it is toward forms of this construction.

#### FOSSIL PLANTS SUGGEST A NEW VIEW OF FLORAL EVOLUTION

The difficulty of tracing backwards the history of a group is comparable to that experienced in finding the source of a river by travelling up from its mouth. Innumerable routes must be fruitlessly followed before the main stream can be distinguished from the lateral branches. More satisfactory results may be achieved by traversing the watershed and noting the general way in which the streams are flowing from the higher levels. So also our present quest may be advanced by studying first the oldest known floras and then trying to trace the general trends of evolutionary change accomplished with the passage of time. It is possible that in this way the floral and vegetative peculiarities of the angiosperms may appear in a new light, and we may be led to make comparative studies of features hitherto neglected.

Recent research (47, 46, 38, 43, 44) shows that the earliest known land plants in several distinct groups, which flourished some 400 million years ago, bore their sporangia at the ends of branches, and there seems to be no direct association of sporangia with foliar structures. Few, if any, plants could be described as possessing foliar sporophylls. Further, some of the oldest forms of Silurian age (47, 18A) possess terminal aggregations of free or united sporangia, which suggest that the sorus may be as old as the strobilus. It is likely that *Aneurophyton* (43), which may well be one

of the oldest known seed plants, bore whorls of pollen sacs at the tips of some of its branches.

As time progressed, the flattened photosynthetic branches of some seed plants became specialized foliar structures with an expanded compound lamina, but the terminal and whorled grouping of their pollen sacs persisted. The fertile branches may have formed part of a large foliar frond as in *Archaeopteris* (59), which was probably, but not certainly, a pteridosperm; however, in other types the fertile and vegetative branches may have remained distinct. The Lower Carboniferous rocks provide us with richer floras and here the forms called *Telangium* and *Scheutzia* by Kidston (37) as well as their contemporary ferns bore whorls or tufts of sporangia at the ends of naked branches. The seeds of these early forms also sprang from the ends of branches, either singly or in groups (1; 37, 464; 9; 10). The fronds and petrified stems from the Lower Carboniferous show that a large number of pteridosperms were then living whose reproductive structures are as yet quite unknown. From the Upper Carboniferous rocks our knowledge is more extensive. In the coal balls we find petrified specimens of the true *Telangium* type (8) where terminal whorls of pollen-bearing sporangia are fused together at the base (Fig. 1, A), while at least two or three other types (Fig. 1, B-D) with the same general construction are known but have not yet received full study (57, 79).

The well known *Crossotheca* (Fig. 1, E) known from moulds and carbonized specimens should probably be described as having whorls of bilocular sporangia borne on a disc-like receptacle terminating a fertile branch (35, 36, 18) though the sporangia have, under the influence of old ideas, been usually spoken of as borne on the margins of a sporophyll (57, 77). The American *Codotheca* belonged to this type of structure. But by this time the reproductive structures were beginning to diverge from the older and central type. Halle (26) has displayed a series, the Whittleseyinae, in which the sporangia of terminal whorls were concrescent and formed a cylindrical synangium varying in form in the different genera (Fig. 1, G, H). Then Halle also studied *Potoniaea* (Fig. 1, F), the probable male flower of the Neuropterids, and here found a large number of free elongated sporangia borne on a cup-like receptacle at the end of a branch. *Potoniaea* shows considerable



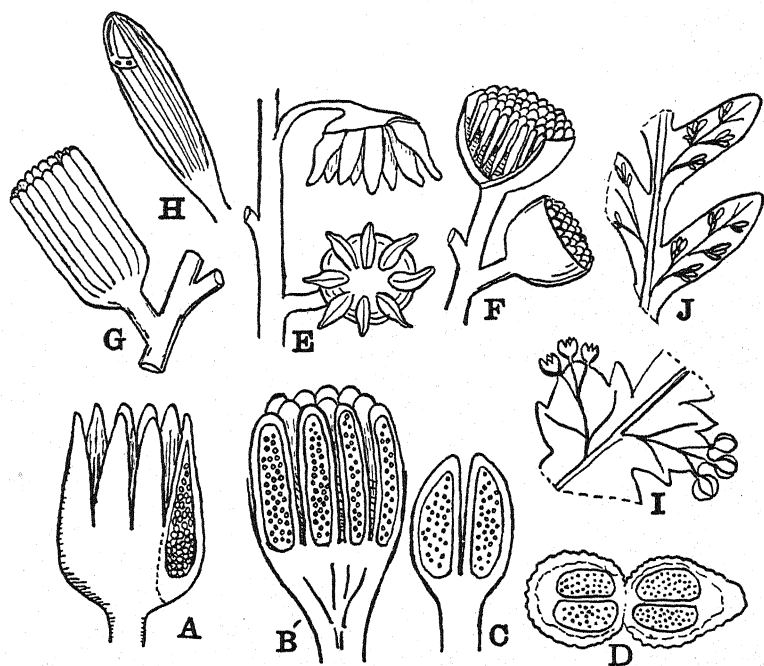


FIG. 1. Typical pollen-bearing organs from the Upper Carboniferous period. A-D, Petrified forms from English coal balls. A, *Telangium Scotti* Benson. B-D, Undescribed forms not yet fully studied. E, *Crossotheca Hoeninghausi* after Kidston. F, *Potomeia adiantiformis* after Halle and Kidston. G, *Whittleseya elegans* after Halle. H, *Aulacotheca elongata* after Halle. I, *Zeilleria avoldensis* after Kidston. J, *Dactylotheca plumosa* after Zeiller. All figures diagrammatic and not drawn to scale.

morphological similarity to the male flowers of *Populus*, *Mollinedia* and other modern forms. In some pteridosperm groups the fertile branches appear to have taken part in frond formation as in the ferns. *Zeilleria* (Fig. 1, I) was one of these and had spherical synangia terminating the main marginal veins of a dissected frond (26), while *Dactylotheca* (Fig. 1, J) had superficial sori of elongated free sporangia (37, 382). Pteridospermous plants are now known to have survived into the Mesozoic (69, 27). Their pollen was produced in elongated unilocular or bilocular sporangia produced in groups or tufts on special branches but showing varied modifications. An expanded receptacle of the *Crossotheca* type is found in *Pteruchus* (69) [Fig. 2, A], but the sporangia may be more or less spread along the fertile limb as in *Lepidopteris* (27)



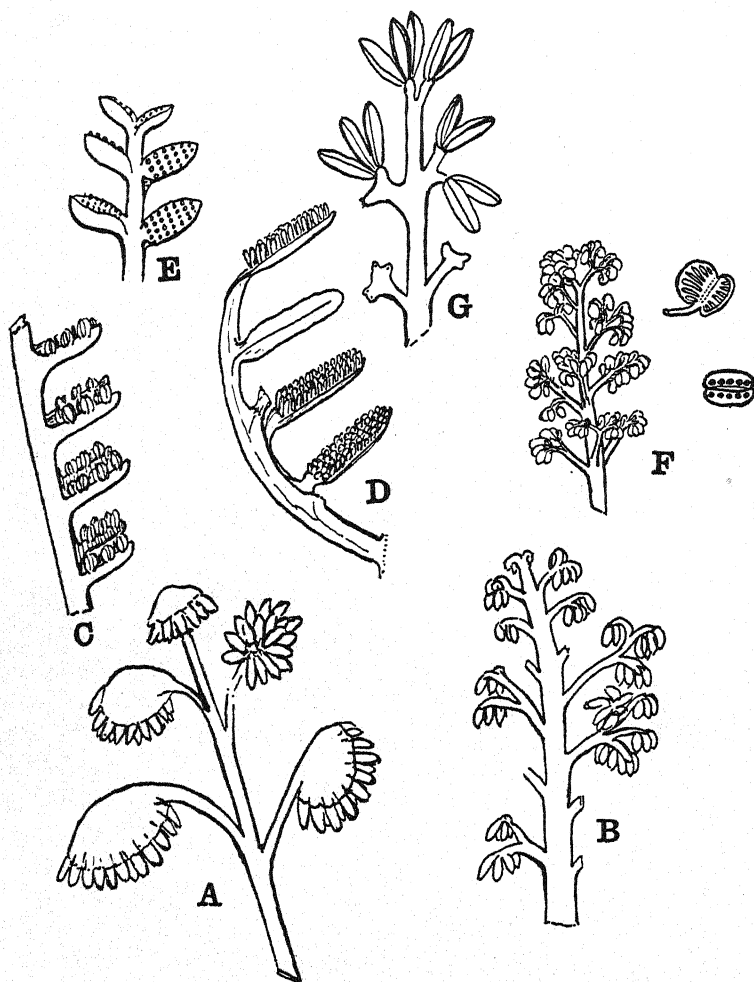


FIG. 2. Pollen-bearing organs from the Mesozoic period. A, *Pteruchus africanus*. B, *Antholithus (Lepidopteris) Zeilleri*. C, *Lunzia austriaca*. D, *Westersheimia pramelreuthensis*. E, *Bennettistemon ambhum*. F, *Hydropteridangium marsilioides*. G, *Antholithus (Caytonia) Arberi*. B, E, F, after Harris; others original. The figures are not drawn to scale.

[Fig. 2, B] and *Lunzia* (40) [Fig. 2, C]. In this way a distinct form of sporophyll is reached. Several little-known Triassic forms, such as *Westersheimia* (40) [Fig. 2, D], show a fuller development of this tendency which leads on through *Bennettiste-*

*mon* (28) [Fig. 2, E] to the Bennettitales. The morphology of the Jurassic and Cretaceous male flowers (76, 77, 48, 49, 67) of this group presents a difficult problem. The well known and characteristic synangia must be considered in relation to *Hydropteridangium* (28, 122) [Fig. 2, F] but the free or concrescent structures which bore them invariably formed a whorl and no tendency towards a spiral arrangement has been yet found. The angiosperm-like anthers of the Caytoniales (68) were produced in groups or singly on branching pinnate structures [Fig. 2, G], which, like those of *Pteruchus* and *Lepidopteris*, may be described as sporophylls, but must have been derived from flattened branch systems. Such structures may have a bearing on the branched stamens found in such plants as *Ricinus*, *Hypericum* and *Calothamnus*, but raise grave doubts as to the propriety of describing all angiospermous stamens as microsporophylls.

The earliest known seeds terminated branchlets [Fig. 3, A-C] and subsequent changes in the position of the seeds are often parallel to changes exhibited by the pollen sac groups. Recent research tends to favor the view of the origin of seeds from a sorus in which a central sporangium only remains fertile (8) while the peripheral sporangia were sterilized to give the integument (and perhaps also the cupule). Many forms of Carboniferous seeds are known [Fig. 3, A-G] but there is very little evidence as to their position on the fertile branches. Grand Eury (23), after many years of field work, expressed the view that most pteridospermous seeds were borne on special branch systems and not on foliage fronds.

It may be significant that the few examples of seeds borne on photosynthetic fronds have been found mainly in the youngest of the Palaeozoic strata (25, 37, 400). The evidence suggests that fronds bearing marginal or superficial seeds were more usual in the Permian period, but the later Triassic forms show a complete separation of fertile and vegetative structures. There can be no doubt that the seeds of the *Corystospermaceae* [Fig. 3, J-L] and *Peltaspermaceae* (69) [Fig. 3, M] were produced on separate branch systems which, having bracts and bracteoles, have been described as single inflorescences. There is no certain evidence of the production of seeds on the margins of a flattened foliar lamina from any Mesozoic rocks. The megasporophyll of *Cycas* has often

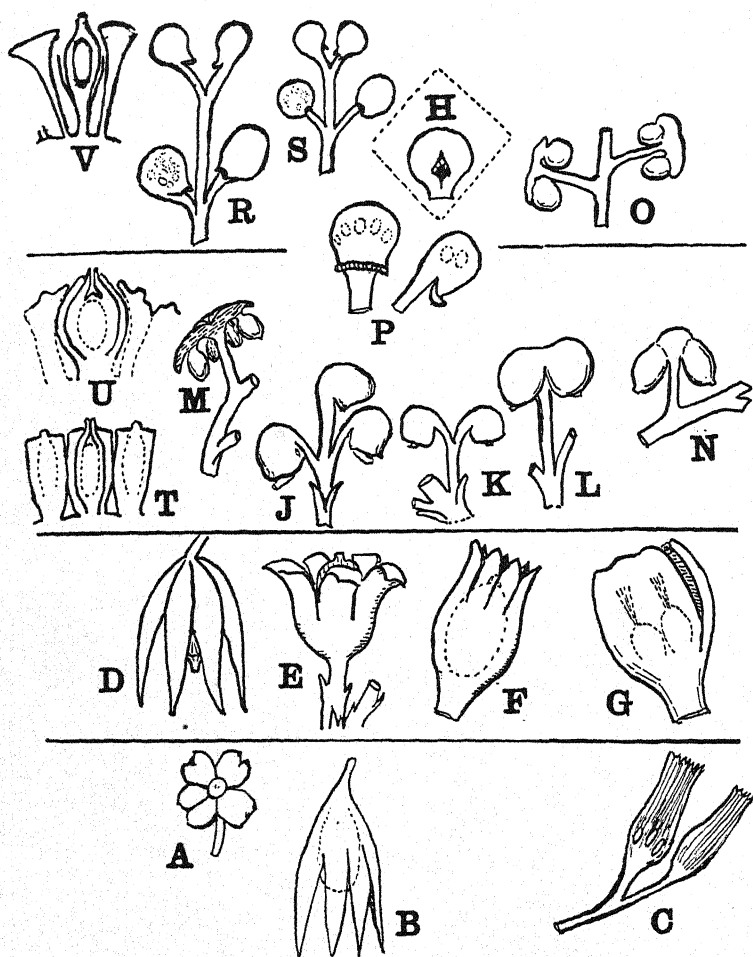


FIG. 3. Seed-bearing structures arranged chronologically to show cupule evolution.

UPPER DEVONIAN AND LOWER CARBONIFEROUS. A, "*Telangium*" *bifidum* after Kidston. B, ? *Archaeopteris* after Arnold. C, *Calathiops Bernhardtii* after Benson.

UPPER CARBONIFEROUS. D, *Sphenopteris striata* after Bertrand. E, *Lagenostoma Lomaxi*. F, *Lagenostoma Sinclairei* after Arber. G, *Gnetopsis elliptica*.

TRIASSIC. J, *Pilophorosperma granulatatum*. K, *P. gracile*. L, *P. geminatum*. M, *Nilssonia incisoserrata*. N, *Lepidopteris*. P, *Caytonia Thomasi* after Harris. T, *Williamsonia Wettsteini*. U, *Vardekloeftia* after Harris.

JURASSIC. O, *Beania gracilis*, probably an early cycadean form. R, *Gristhorpia Nathorsti*, with closed ovary and large stigma. S, *Caytonia Sewardi*, closed ovary and small stigma. V, *Bennettites*, and *Williamsonia* with much reduced cupule. H, Hypothetical early angiospermic carpel formed from two concrescent cupules with lateral stigma.

All figures diagrammatic and not drawn to scale.

been used in discussions on carpel morphology, and the fossil *Cycadospadix* (*Palaeocycas*) has been compared with this structure, but Florin (22) has shown that this structure differs considerably from the modern *Cycas* and it may be here remarked that no fossil specimen has ever been found with an attached seed. On the other hand, the Triassic and Jurassic structures named *Beania* (Fig. 3, N, O) suggest that the flattened megasporophyll is not necessarily a primitive type of structure in the cycads.

The Jurassic Caytoniales seem to show how angiospermy actually came about in a group of plants, descended from pteridosperms of the *Pilophorosperma* type, but quite distinct from and probably contemporary with the earlier angiosperms. A Triassic member of this group (29) was a gymnosperm (Fig. 3, P) for the pollen grains reached the micropyles of the seeds. The later Jurassic members (Fig. 3, R, S) were angiosperms with the pollen grains adhering to a stigma and never reaching the ovules directly (68). The ovary wall must here be related to the cupule of the Palaeozoic seeds, and a number of seeds was produced within it. Thomas has suggested that the carpel wall of the angiosperms may represent a pair of concrescent cupules (70, 72) and that the possible origin of the stigma should be considered in the light of these ancient forms (72). In earlier papers of this author it was assumed that the current morphological interpretations of stamens and carpels were correct, but subsequent consideration of the foundations of the classical theory (71) have caused important modifications of his earlier views; however, the idea that the angiosperms, Caytoniales and Bennettitales arose from a common ancient plexus is still maintained.

The present position is that the fossil record seems to provide material for a completely new view of the morphology of the closed ovary and stigma. This view needs to be tested without preconceptions by comparative studies of modern forms.

The Mesozoic Bennettitales diverge widely from the angiosperms in the construction of their ovuliferous parts, but must be considered in relation to floral morphology (1A). In this group we find reproductive structures closely aggregated at the top of a longer or shorter axis, showing no indication of strobilar structures in their early stages (76, 178, 63, 431). Some genera show unisexual and others bisexual aggregates, often with a number of

lanceolate structures like a perianth. The upper part of the fertile axis bears a very large number of appendages packed together to form a spherical or conical mass, and differentiated into stalked ovules and sterile interseminal scales. Some of the older forms (Fig. 3, T) from the Trias (41) give strong indication that the interseminal scales represent aborted ovules and certain later examples (28, 112, 63) (Fig. 3, U) support this interpretation. The so-called female strobilus would then represent a terminal tuft of ovules in which little or no trace of regular spiral arrangement is discernible. But the structure of these ovules shows strong affinities with the pteridosperm seeds, and it is known that the earlier forms possessed cupular envelopes which became much reduced in the later forms. We thus get no approach to the expanded foliar carpel of the older authors, but we have to deal simply with a mass of gymnosperm ovules at the apex of a shoot. If this is the true interpretation of a flower-like structure which lasted for a very long time, it seems possible to apply it to certain angiospermous flowers which show carpels crowded on the floral axis. It is at least possible to consider that the earlier ancestors of some flowering plants may have had a terminal aggregate of short stalks each bearing a *pair* of cupules enclosing two or more ovules which by the concrescence of the cupules gave rise to closed ovaries. Furthermore, it is possible that the process which resulted in the production of bisexual bennettitalean flowers from the unisexual groups of reproductive structures found in the older pteridosperms may well have given rise also to the bisexual flowers of the angiosperms. The nature of this process awaits further discoveries in the field of recent taxonomy, cytology and genetics, as well as the investigation of the much neglected physiology of reproduction.

In concluding our historical survey of the reproductive structures of megaphyllous seed plants, we cannot avoid certain conclusions:

(a) That while no support can be found for the classical views of floral morphology, the consideration of all the known forms in their chronological sequence makes inevitable a new concept of floral structure.

(b) The evidence derived as we progress from a period some 400 million years ago to the present day provides material for a consistent and physiologically possible theory of floral evolution which accords with what we know of the Cretaceous and early Tertiary angiosperms and seems applicable to the flowers of to-day.

(c) The flower seems to have commenced as a sorus or tuft of sporangia terminating a branch before any specialized foliar structures had been evolved.

(d) Concrescence of the elongated pollen-bearing structures was an early feature while the apex of the stem often appears expanded to a disc-like receptacle. Several Coal Measure pteridosperms had male flowers morphologically similar to those of *Populus* but their anthers were uni- or biloculate.

(e) The concept of the stamen as a complete microsporophyll seems untrustworthy.

(f) Seeds arose singly on branch endings, but soon show aggregations. Single seeds or groups became enclosed in an envelope, the cupule, which covered them more and more completely as time went on and often became recurved.

(g) Pairs of cupulate seeds sometimes grew on short branches of an "inflorescence," and in the early Mesozoic may show a partial fusion of the two cupules.

(h) In one group the cupules became closed and the pollen grains were deposited on their papillate margins instead of reaching the micropyles directly, so effecting a change from gymnospermy to angiospermy.

(i) The carpels of flowering plants may then possibly represent two fused cupules rather than a single foliar structure. The more primitive carpels would thus be small almost spherical structures with an extended stigma arising on their ventral side (Fig. 3, H).

(j) In different groups and at different periods cupulate seeds or seeds enclosed in an ovary appeared in the center of the receptacle or disc bearing pollen sacs.

From the fossil evidence a picture of floral evolution can be constructed in which almost every stage is represented by an actual plant structure dating from about the period when we should expect it to occur.

It remains to be seen whether these concepts are fully applicable to modern genera, but they have obvious advantages over the classical views which involved the postulation of an ancestral gymnosperm differing from any known plant, and a course of evolution for which there is no evidence. Botanists may be reluctant to give up views which have been passed on by so many generations of investigators, but if so they must provide the classical theory with objective foundations. For instance, they must provide good reasons for believing that modern flowers have arisen by the condensation of an elongated floral axis and not from a terminal sorus as has been suggested above. However, we have several independent sources which ought to provide means of testing the theories

of the comparative morphologist as to the nature of primitive flowering plants and thus a wide field is open for future investigation.

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## APPENDIX

*Geological Time Scale*

| <i>Period</i>                           | <i>Approximate age<br/>in millions<br/>of years</i> |
|---|---|
| Upper Tertiary { Pleistocene            | 30  |
| { Pliocene                              |   |
| { Miocene                               |   |
| Lower Tertiary { Oligocene              |   |
| { Eocene                                | 60  |
| Upper Cretaceous { Senonian             |   |
| { Turonian                              |   |
| { Cenomanian                            |   |
| Lower Cretaceous { Albian—(Patapsco)    | 120   |
| { Aptian                                |   |
| { Neocomian (Wealden, Patuxent)         |   |
| Jurassic                                | 150   |
| Triassic                                | 180   |
| Upper Permian                           | 210   |
| Lower Permian or Permo-Carboniferous    | 240   |
| Upper Carboniferous                     | 270   |
| Lower Carboniferous                     | 300   |
| Upper Devonian                          | 360   |
| Middle Devonian                         | 390   |
| Lower Devonian                          | 420   |
| Silurian                                | 450   |
| Lower Cambrian with first-known fossils | 600   |

## EXPLANATORY NOTES

angiospermy: the condition of ovules being borne within a closed structure, the ovary. Applies to all modern seed plants except the Ginkgoales, Cycadales, Coniferales and Gnetales which are gymnosperms.

coal-balls: calcareous nodules containing petrified patches of vegetable debris from the marshes and swamps of the closing stages of the Carboniferous period (Seward).

concrecence: a growing together.

cupule: a free sheathing structure from the peduncle investing one or more seeds (Oliver & Salisbury).

epigynous: refers to flowers whose stamens stand on the pistil, apparently above the ovary.

gymnospermy: the condition of ovules being borne, not within a closed ovary, but uncovered. See angiospermy.

homoxylous: plants with wood of uniform structure and which (like the pines) contains no vessels.

hypogynous: refers to flowers having stamens inserted below the ovary.

megaphyllous } in evolutionary studies plants have been divided into the  
microphyllous } small-leaved (micro-) such as mosses and lycopods, and the large-leaved (mega-).

perigynous: refers to flowers having stamens on a rim-like expansion of the receptacle or on the perianth. Intermediate between hypogynous and epigynous.

pteridosperm: prehistoric fossil plants, fern-like in foliage but with gymnosperm-like reproductive organs.

receptacle: the portion of a flower axis which bears the floral organs.

ring-porous wood: wood in which the pores (vessels) of one part of a growth ring are in distinct contrast in size or number (or both) to those of the other part.

scalariform vessel: a vessel having elongated perforations or thickenings showing a ladder-like arrangement, like those of woody elements of ferns.

sorus: a cluster of sporangia in ferns.

sporangium: a sac producing spores within it.

sympetalous: with united petals. Generally regarded as an advanced type.

synangium: an aggregate of united spore-sacs formed either by the union of several distinct sporangia or by the development of separate chambers in a single sporangium.

strobilus: a cone-like structure made up of overlapping scales (sporophylls) bearing reproductive organs.

## PLANT TISSUE CULTURES

PHILIP R. WHITE

The term "tissue culture" is too well known to the scientific public through the work of Harrison (56-59), Burrows (10), Carrel (11-15), Fischer (31-36), Erdmann (25, 26), Lewis and Lewis (78-80), and others to need definition. In general, it has been used in such work to designate preparations in which somatic cells of a single type or a restricted number of types (fibroblasts, osteoblasts, epithelial cells, etc.), isolated from an animal body, have been kept growing for more or less extended periods of time, *in vitro*.

The concept of potentially unlimited growth as a *sine qua non* of a tissue culture is, in general, adhered to by Carrel and his school, but rejected by Lewis and others. Likewise, the concept that a tissue culture should be of a single type of cell and should undergo only limited differentiation is generally adopted by Carrel (13, 15), but must be rejected in the application of the term to the "organule" cultures of Fischer (31, 32, 34), Fischer and Parker (37), Fell (29, 30), *et al.* In view of this divergence of opinion, even in the animal field, as to just what constitutes a "tissue culture," and in view of the comparative newness of the term in the working vocabulary of most botanists, it seems desirable to begin by setting forth a definition for use in the following pages. A plant tissue culture will hereafter be considered to be *any preparation of one or more isolated, somatic plant cells which grows and functions normally, in vitro, without giving rise to an entire plant.* Under such a definition, spore cultures, cuttings, and cultures of whole embryos will be excluded, but a limited amount of differentiation will not be considered as automatically excluding a particular culture from this category. It is realized that such a definition may raise objections in some minds, and suggestions for its improvement will be welcomed. The author is inclined to concur in Carrel's concept that unlimited growth is a necessary criterion to be included in a proper definition, but since such an interpretation would restrict the literature of the field to only a very few papers, it will not be insisted upon at present. Nevertheless, it should be kept in mind that *unlimited survival* and growth has been the *aim*

of most of the work in both the animal and plant fields, and that one of Carrel's chief contributions to the field of animal tissue cultures was the development of a nutrient which would permit this aim to be attained (14).

Until recently, active interest in the subject of plant tissue cultures has been restricted almost exclusively to Germany, and publication has been mostly in non-botanical journals. For early discussions of the problem the reader is referred to the résumés of Börger (7), Küster (70, 71), Lamprecht (76), Schneider (107) and White (123), and the historical introductions to the memoirs of Gautheret (44) and Scheitler (104).

Certain phases of botanical research not falling strictly in the category of tissue cultures have, nevertheless, been important in laying the foundations of technique and knowledge of tissue behavior necessary for success in the more specialized field, and should be briefly mentioned. Studies in tissue repair in injured plant organs (Vöchting, 119, 120; Hanstein, 55; Jaeger, 62; Küster, 68; Schilling, 105; La Rue, 72; Reiche, 98; Lopriore, 81), of callus formation and abnormal proliferation (La Rue, 73; Němec, 89; Stingl, 111; Haberlandt, 52, 53; Okado, 90; Dale, 20; Küster, 69; von Schrenk, 108; Winkler, 132), and of wound healing in general (Haberlandt, 52, 53; Brieger, 8; Lamprecht, 75; Miede, 86; Olufsen, 91), have added considerable information to our store of knowledge of the conditions necessary for tissue proliferation. The cultivation of isolated embryos (Andronescu, 1; Arnaudov, 2; Tukey, 116, 117; Dietrich, 23; Essenbeck u. Suesenguth, 27; Buckner and Kastle, 9; Hannig, 54; Stingl, 110; White, 124) and the aseptic cultivation of whole plants (Combes, 18; McMillan, 85; Klein u. Kisser, 64; Bobko, 5; Hatch, 60; Weissflog, 122; Gerretsen, 45) have aided in determining the necessary environmental conditions and the experimental techniques adapted to such work. The extensive literature on the rooting of cuttings has likewise contributed much to our background.

While it is thus possible to mention a great deal of literature collateral to the subject, that referring strictly to plant tissue cultures, as such, as defined here, is quite meagre. Any treatment of the subject must begin with the work of Haberlandt (46-53), reported during the first third of the present century. Haberlandt clearly formulated the problem in 1902 when he said (46):\* "So

\* Reviewer's translation.

far as I know, there has been, up to the present, no well planned attempt to cultivate the isolated vegetative cells of higher plants in suitable nutrients. Yet the results of such cultures should throw many interesting side-lights on the peculiarities and capacities of the cell as an 'elementary organism'; they should bring into evidence the reciprocal relationships and many-sided influences to which the individual cells of a multicellular organism are subjected." This is, so far as the present writer is aware, the first formulation of the tissue culture idea, antedating by several years the first work in the field of animal tissue cultures (Harrison, 1907 (56), Burrows, 1910 (10), Carrel, 1911 (11)). Haberlandt attempted at the very outset to cultivate single cells, using palisade and medullary parenchyma, trichomes of various types (glandular hairs, stinging hairs, stamen hairs), epidermal cells, etc. (46). Although a perusal of this and subsequent papers makes it evident that he and his pupils grasped very well the nutritional problems involved, they seem not to have appreciated the important growth-restricting effects of differentiation, especially as regards the deposition of cell walls. The experimental results obtained were not encouraging. These investigations were continued in Haberlandt's laboratory over a long period of time, by Bobilioff-Preisser (4), Lamprecht (75), Thielmann (112-114), Thielmann and Bérziñ (115), and others, and independently in other laboratories the same sort of approach has been used by Pfeiffer (95, 96), La Rue (72-74), Scheitler (104), Ulehla (118), Schmucker (106), Kemmer (63), Börger (6), Czech (19), Winkler (132), and Kunkel (67). These authors never obtained definitely demonstrable, continued proliferation, and in 1927 Küster reviewed this work with the statement that (71)\* "never to this day have isolated (plant) cells been brought to reproduce their tissues; all attempts have given the same negative results."

Molliard (88), Dauphiné (21), Reching (97), Scheitler (104), and Smith (109) have attempted to grow small fragments of various tissues, especially pieces of embryos, with little success. The only work of this sort which appears to have been at all successful is that of Behre (3) with tissues of *Drosera* and certain unpublished work (1936) of La Rue (74), which cannot as yet be properly evaluated.

\* Reviewer's translation.

From a careful study of these papers, the present reviewer feels justified in saying that up to 1936 only two groups of work in the field have given real promise of success. These two have taken their initial inspiration from the laboratories of Haberlandt (Berlin) and Molliard (Paris), and have dealt, respectively, with apical and with lateral meristems. Because it can be treated more briefly, the second mentioned work will be discussed first.

In 1935 Gautheret published an extensive memoir (44) entitled "Researches on the cultivation of plant tissues," in which some 50 pages are devoted to a discussion of experiments on the cultivation of cambium removed from large trees of various species. The same work was summarized briefly in an earlier paper (43). Fragments of cambium a centimeter or more on a side were excised under aseptic conditions and placed on cotton or gelatin saturated with a nutrient solution. A slow proliferation was obtained giving rise to loose pseudo-parenchymatous outgrowths resembling the thalli of *Pleurococcus*. Growth continued over several months. These proliferations were similar to those described in regenerating tissues by Hanstein (55) in 1882. Histological preparations showed extensive modification of structure, not only of the surface layers but of more deep-seated tissues as well, with formation of an undifferentiated tissue resembling, at the exposed surface, that occurring in lenticels (Hanstein, 55) and, in the deeper tissues, that formed from proliferating pericycle and medullary rays or in the healing of internal wounds, as observed by Jaeger (62). Mitotic figures occurred frequently. The conditions employed did not permit indefinitely prolonged growth, so that this work does not fulfill that criterion of a tissue culture, and it is rather uncertain whether the changes occurring can be interpreted as those normal to a healthy tissue released from the influence of surrounding tissues, or merely those characteristic of injured cambium and cortex, left *in situ*. Moreover, only a small part of each explant took part in the growth, the major portion remaining dormant or becoming necrotic, so that it is uncertain if these cultures actually drew any of their material for growth from the nutrient supplied. The work will have to be greatly extended before it can be properly evaluated. Nevertheless, the result is promising and is important as representing the *only* case in the entire literature of the subject

\* Reviewer's translation.

which would fulfill Carrel's second requirement, of *undifferentiated* active growth. In the strict sense, this is thus the only work reported to date that can even tentatively be called a "tissue culture" of plant material, and even this, as pointed out above, is a somewhat doubtful case. Further work in this direction will be followed with great interest.

This study of Gautheret represents the only work with the second category of tissues mentioned above, lateral meristems (cambium), which gave promise of success. The other, and in the present writer's opinion more promising and much more extensively developed approach to the tissue culture problem, has been that with isolated terminal meristems—stem-tips and root-tips.

The culturing of stem-tips is mentioned briefly by Robbins (100) and is treated in more detail by White (127). Using stem growing points of *Stellaria media*, the latter obtained considerable growth and differentiation, with apparently normal photosynthesis. The complex of conditions employed was not satisfactory for unlimited growth, and the normal mode of differentiation shown by these cultures suggested the behavior of cuttings rather than of tissue cultures, so that the work need not be treated in detail.

The cultivation of root-tips has been much more widely attempted and has given more satisfactory results. Heidt (61) and Felber-Pisk (28) studied the behavior of isolated roots in non-nutrient media or in air, and obtained only a very limited growth. Mayer (84) employed a great many different nutrient combinations, but obtained unsatisfactory results, probably due to excessive handling of the cultures with concomitant trauma. Really promising results with root-tips, however, were obtained by six authors, Kotte (65, 66), Robbins (100, 101), Robbins and Maneval (102, 103), Malyshev (82, 83), Gautheret (39-42), and White (124-126, 128-131). This work will require more extensive treatment.

In 1922 three papers on the cultivation of excised root-tips in nutrient media, representing independent work, appeared almost simultaneously: two by Kotte (65, 66) working under Haberlandt in Berlin, the other by Robbins (100) in the United States. Kotte grew apical and sub-apical fragments of *Pisum* and *Zea* roots on various media. He studied the nutrition of such root fragments, examining their requirements as regards sugars, organic nitrogen sources (amino-acids, peptones, tissue extracts), and inorganic



salts. He also studied their behavior toward light and gravity, the effect of size of initial fragment, of position of fragment in the root, of age of tissue, the phenomena of growth polarity, etc. He obtained considerable but not indefinite growth, some differentiation, but no suggestion of the formation of stem primordia. His best results were obtained with *Zea* roots in tubes of agar containing a Knop solution plus 1% dextrose with the addition of any one of four accessory materials which were, in descending order of satisfactoriness: 1) Liebig's meat extract; 2)  $\alpha$ -alanine; 3) a mixture of peptone, asparagine, alanine, and glycine; 4) a pepsin-diaxase digest of pea seeds. Such cultures grew well and branched actively for some time. Sub-cultures were not attempted and Kotte has published no further work on this subject.

In the same year Robbins, working independently, published (100) similar results. He grew roots of *Pisum*, *Zea* and *Gossypium*. He found a liquid medium somewhat more satisfactory than a solid one and studied the effects of several different sugars, concluding that dextrose was satisfactory for these plants. Roots were carried through three transfers (six weeks) but eventually died. In a second paper (101) the effects of various organic nitrogen sources were studied. The amino-acids were consistently found to be either detrimental or without effect. The best results were obtained with an autolyzate of yeast. This was studied at several concentrations. The same author and Maneval (102) extended the study to include tissue extracts and different concentrations of the nutrient ingredients, and in 1924 studied (103) the effect of light in the presence or absence of yeast extract. They concluded that moderate illumination was desirable. Many different sorts of plants were studied. All species, with the exception, curiously enough, of *Lupinus albus*, gave fairly satisfactory results. Some cultures were carried through ten passages (150 days), but eventually showed markedly decreased growth rates. These four papers (100-103), together with Kotte's two (65, 66), established a fairly secure basis on which to build, giving distinct promise of success.

Chambers' experiments (16, 17) in growing squash root-tips in hanging drops, in which he reported migration of cells as in animal fibroblast cultures, have not been confirmed, and were probably erroneously interpreted (see 123, 124).

In 1932 Malyshev, using a technique similar to that of Kotte, succeeded in carrying root cultures through 13 passages (83). He does not give details of growth rates, but made certain important observations on the differing carbohydrate requirements of different plants.

The major part of the history of this phase of the subject in the last ten years is, however, mostly the work of Gautheret and of White. In 1932 and 1933 Gautheret, who has already been mentioned in another connection, grew a number of sorts of roots and root fragments in various nutrients (39-42). An extensive account of this and other work was published in his memoir of 1935. All of his roots ceased to grow after a certain period varying from 10 to 100 days, and the author concluded that unlimited growth is not possible. Like many other authors, he has recourse, as an explanation for this failure of isolated tissues to grow indefinitely, to the theory of "hormones" provided by the parent plant, essential for growth and not formed in these tissues. Gautheret made a number of contributions to the technique of such cultures. In the opinion of the present reviewer, an important contribution was the demonstration that, although cystein-HCl is toxic at concentrations corresponding to those used by Robbins, Kotte, and other earlier workers in studying the effects of amino-acids, it is, nevertheless, markedly stimulating at lower concentrations, of  $10^{-6}$  or less (44). Since neither unlimited growth nor undifferentiated growth was obtained, the results are, however, merely suggestive of certain potentially fertile methods of approach. Dauphiné (22) and Galligar (38) have made somewhat similar cultures.

In 1931 White began a series of studies in the general field of plant tissue cultures (123-130). In an introductory paper (123) the history of the subject was reviewed in detail. A brief paper (124) on the cultivation, in hanging drops, of three types of meristems: root-tips, embryos, and seed primordia, appeared in 1932. So far as the writer is aware, the only other paper in which attempts at cultivation of the last-named type of tissue are discussed is the unpublished dissertation of Moebius (Leipzig) (87). All three sorts of tissue gave distinct promise of success; only one, root-tips, has been followed up in detail, however. In 1933 appeared the paper on stem-tips (127) already mentioned. The cultivation of root-tips of *Triticum* was taken up in detail in 1932,

with a study of the effects of light, temperature, H-ion concentration, aeration, solution-volume, and presence or absence of dextrose or yeast or both (125). In 1933 the inorganic nutrient was carefully studied (126) with respect to each of its constituent ions, and a satisfactory formula developed. A very rapid and apparently normal growth was obtained for the short periods studied (two weeks only) in the best complex tested. The chief points of importance to be found in these papers are: the sharply-defined *maximum* temperature for growth (ca. 28° C.), the necessity for an *acid* solution (pH 5.0 to 5.5), in which respect these cultures differ from those of animal tissues which require a neutral or alkaline medium, the very low maximal as well as optimal concentration of phosphate (ca. 0.1 millimol as  $\text{KH}_2\text{PO}_4$ ), the extreme sensitiveness of such cultures to absence or too low concentration of iron, the sharply-defined optima for calcium and potassium concentrations, and the relative indifference of the tissues studied to variation in anion concentrations. The nutrient developed in this work has been repeatedly compared with other well-known nutrients and found superior for such cultures (unpublished results). A brief note (128) on the effect of -SH compounds on root cultures, and one on the relative merits of liquid and solid media (129) likewise appeared in 1933.

Indefinitely-continued growth of such cultures was not obtained by Kotte, Robbins, Chambers, Malyshev, or Gautheret, and more or less elaborate theories, such as that of Miede (86), have been evoked to explain this fact. The most widely accepted of these theories: that the plant must contain substances necessary for growth, which are irreplaceable and whose synthesis by isolated roots from the constituents of the culture medium is impossible, is perhaps best expressed by Gautheret (44, pp. 109-110, 1935). The arrest in development would, according to this theory, be the result of a disturbed equilibrium arising from the isolation and causing a complete change in the nutrition of the root. This has been the point of view of most of those who have worked in the field within recent years, and was justified by much of the data available until recently. In 1934, however, White (130), using tomato root-tips, a nutrient and culture method based on those developed in earlier papers (125, 126), with carbohydrate changed from dextrose to sucrose as required by tomato (unpublished re-

sults; compare Malyshev, 82), and employing certain slight modifications in method of preparing the nutrient, succeeded in carrying roots through more than 50 passages. A mean growth rate of ca. 5 mm. per culture per day (a quite normal value) was maintained, and a total *potential* tissue multiplication of the order of  $10^{40}$  was obtained. The *actual* measured linear increment of a single isolated root-tip during one year of culture *in vitro* was 15,500 times the initial length, with 35,400 branches. This same root has now, at the present writing, been carried through about 160 passages (more than three years), has made a total measured increment exclusive of branches of nearly 2 miles, and is still growing entirely normally. The theory of irreplaceable essential substances expressed by Gautheret (44) and suggested as a possibility by Robbins (101) and others is evidently unnecessary, although "accessory substances" of an as yet unknown nature have been regularly supplied to these cultures in the form of a soluble extract of .01% of dried brewers' yeast (Harris). Since, however, Osborne and Wakeman (92) have shown that such an extract is free from demonstrable protein, these "accessory substances" cannot be very complex and should be capable of identification. In any case, they represent only a very minute fraction (less than 1/10,000) of the nutrient. An important feature of this work is that the medium used, unlike the media employed in animal tissue cultures, contains only this one unknown. All other constituents of the nutrient are known and can be varied at will.

Certain characteristics of these cultures are worthy of note. In routine work the initial explant has been regularly a piece 10-15 mm. long, including the apical meristem and some elongating tissue (130). This normally floats on the surface of the nutrient (125). Sometimes roots which at first sink to the bottom will subsequently come to the surface, but the growth rates and general appearance of the cultures are almost invariably better in those which have floated throughout the culture period (125). These must be considered to represent the normal type. This behavior of healthy tissues is probably due to occluded respiration gases. The excised root elongates as does a root in the soil, without any apparent abnormality except that, contrary to Kotte's findings (65, 66), it does not react geotropically. Five to 10 mm. of the tip of a growing culture may bend downward somewhat, but this curvature is

always removed in the region of elongation, so that a root grows horizontally throughout all but the terminal centimeter and continues straight as long as the restricting space allows, later curving around the inner surface of the flask. Whether failure to bend permanently geotropically is due to loss of reactivity, or merely to a balance between the downward geotropic tendency and the upward chemotactic reaction toward the oxygen gradient in the nutrient, or to the low specific gravity of the tissue which, in the absence of any firm point of attachment, prevents the geotropic reaction from being effective, cannot at present be said. Growth rates are somewhat variable but in general average an increase of about 5 mm. (one-half to one-third of the culture's original length) per day. A culture thus doubles in volume in the first 48 to 72 hours. This figure is comparable to that considered normal for animal tissue cultures (15). Individual growth rates as high as 40 mm. per day have occasionally been recorded, and one group of cultures under unusually satisfactory conditions averaged 13 mm. per culture per day for an entire week (unpublished data). Cultures usually begin to branch from the base on the third or fourth day, and at the end of a week will frequently have 30 or 40 branches, each one capable of being used as a sub-culture. The roots themselves are entirely normal in color; the root-cap is characteristic in its form and in its manner of sloughing off (124). The cells reported by Scheitler (104) as simulating migrating cells (compare Chambers, 16, 17; Pfeiffer, 96; and White, 124) were apparently sloughed off root-cap and root epidermis cells. The main portion of the culture, contrary to the observations of Malyshev (82) (see Gautheret, 44, p. 99), maintains a constant diameter. If branch roots of small diameter are used as sub-cultures, they very quickly enlarge to this normal diameter as they grow. Because of this fact, linear increments are quite as satisfactory measures of tissue increase (125, 129, 130) as are dry weights. Cultured roots seldom exceed this norm, and when they do, are evidently unhealthy. Such excessively thick cultures do not grow satisfactorily. It is, therefore, clear that secondary thickening is not a characteristic feature of such isolated roots. Nevertheless, complete vascular strands containing all the primary elements are regularly formed (124, 130), although it would seem improbable that they perform either of their major normal func-

tions of conduction and support. No interfascicular cambium or pheloderm is laid down, but proliferation of the pericycle to form secondary growing points (branches) goes on normally. When a piece of root containing no apical growing point is cultured, callus is formed in considerable quantity at the distal end but not at the proximal (Kotte, 66; compare Vöchting, 120), and when branches are formed on such a piece their insertion on the root is characteristically oriented. The polarity of growth, as was pointed out long ago by Vöchting (120), is thus maintained. The root-cap cells contain normal quantities of starch and the older cortical cells at the base of cultivated roots, likewise, become loaded with starch. The development of these cultures, so far as it goes, appears to be quite normal both in quality and quantity. But, although *growth* is potentially unlimited (130), *differentiation* is not. These are typical rootlets, but in the absence of secondary thickening, cambium, pheloderm, etc., even in cultures maintained for months without transfer (130), they cannot be called "roots" in a complete sense. And since no stem-tips are formed, they are certainly not plants.

The question arises, are they "tissue cultures"? Strictly speaking, they are not. They are "organ cultures." Strictly speaking, "tissue cultures" of plants have never been obtained. Gautheret's cambium cultures (44), mentioned before, appear to have satisfied the criterion of undifferentiating growth. White's cultures have satisfied the criterion of unlimited growth. But both criteria have never been satisfied in any single instance reported to date. The consideration of these root cultures in a paper on tissue cultures must be justified on the basis of their aim, and the fact that the method of approach is certainly that which must be employed if tissue cultures are to be obtained. Furthermore, it is the writer's belief that there is a growing feeling among animal tissue culturists that "differentiation" is a matter of environmental conditions as much as it is of internal factors, and that the undifferentiating pure-line cultures of Carrel (13), Ebeling (24), Fischer (35, 36), Parker (94), and others have come to be considered the normal type only because of the aims in mind. Thus Parker, using a pure-line culture of blood monocytes, but replacing the usual solid medium by a liquid medium similar to that used by White and Robbins, caused these pure-line cultures to differentiate into

a capillarogenic tissue (93). The work of Carrel, Ebeling, and others has shown that heart fibroblasts, when grown in pure culture, maintain an undifferentiated behavior indefinitely. Cartilage cells derived from the embryonic eye of the chick, in pure culture, lose their ability to produce hyaline sheath substance, developing as an undifferentiated epithelium-like tissue quite different from normal cartilage (Fischer, 35). Cartilage from embryonic bone, freed from perichondrium, does not grow at all (34). But when a pure culture of heart fibroblasts and a pure culture of epitheloid tissue derived from cartilage are grown side by side in the same medium, at the point of contact a new tissue, actively growing typical cartilage, arises and can be carried on indefinitely (34). This is then no longer a "tissue culture" in the strict sense of being a pure-line culture of a single type of cell, and cartilage is not, strictly speaking, a "tissue." This point of view has been presented by Parker, Fischer and Parker, Fischer, Fell, and others, and shows the absurdities that result from trying to draw too sharp a line between what is and what is not a tissue culture.

Work with root cultures is being continued in an effort to simplify the method and make it more reliable, and these results are being applied to practical problems of physiology and pathology. One of the unfortunate features of the method is the rigid control necessary if satisfactory results are to be obtained. Such isolated plant organs are extraordinarily sensitive. Many of the important cultural variables have been studied in some detail (temperature, pH, etc.), but others have apparently even eluded identification. The sensitiveness of such roots is shown, for example, by the fact that increasing the total salt concentration of the solution used from 2.6 millimols (the optimal concentration) to 10 millimols, both extremely low absolute concentrations, will reduce the growth obtained by about 60% (unpublished results). A change of maintained temperature from 27° to 30°, a difference of only 3° but across a critical range, will reduce the growth rate of wheat root-tips from the normal average value of about 5 mm. per day to almost nil (125). Decreasing the Fe-ion concentration from .003 milliequivalents to .002 milliequivalents will decrease the tissue yield by 50% (126). The roots are even more sensitive to such variants as the cystein-HCl studied by Gautheret (44). Moreover, there appears to be marked specificity as regards carbohydrate re-

quirements, since tomato requires sucrose, being unable to utilize dextrose under the conditions studied, while wheat gives excellent results with dextrose. Efforts to increase the phosphate concentration so as to make possible buffering of the solution have regularly proved fatal. Failure to control properly some one or more of these and possibly unknown factors is doubtless responsible for the checks encountered by other workers, and it can not be over-emphasized that the nutrient formulae, cultural conditions, etc., as published, can with certainty be applied *only* to the species of plant described.

As Haberlandt foresaw (46), the cultivation of excised plant tissues has thrown many interesting lights on the behavior of certain groups of cells even though single cells have not been successfully isolated. The *apparent* dependence of chlorophyll-free tissues on moderate illumination, as reported by Robbins and Maneval (103), White (125), and Felber-Pisk (28), but denied by Malyshev (82), is interesting and will require further verification. The necessary character of iron, usually thought of as merely a catalyst for chlorophyll formation, for the proper maintenance of a chlorophyll-free tissue (126), is likewise interesting, but quite in keeping with Warburg's concept of the relation of iron to respiration in general (121). The fact that tomato roots are able to utilize sucrose under the conditions studied, but can not utilize dextrose, indicates that either sucrose, contrary to earlier opinion, can enter the cells as such and is there utilized directly or broken down to materials other than dextrose and levulose, or else that roots secrete into the medium some hydrolyzing agent giving rise to materials other than these hexoses. The proved unlimited capacity for growth of such cultures (130) obviously sets aside the concept of indispensable and *specific* correlation hormones, since the only possible source of such hormones has been the thermo-stable, apparently non-protein (Osborne and Wakeman, 92) filtrate of a plant (yeast) taxonomically and physiologically widely separated from the cultured tissue. These and many other interesting facts have been brought to light by the studies made to date, and give some indication of the potential value of this method of approach. It is to be noted that every factor in the environment, with the exception of the yeast extract, is of known character and can be controlled and varied at will, so that all sorts of problems of nutrition



and of other phases of physiological behavior can be approached in this way. The method represents a powerful new tool for such studies. Unfortunately, perhaps because of the sensitiveness of such cultures and the need for rigorous control if results satisfactory for physiological studies are to be obtained, the method has not as yet taken a prominent place in the plant physiologists' armamentarium. It has received somewhat more attention from the pathologists and has been used as a means of maintaining pure-line cultures of obligate parasites (viruses, 131), and in the study of nodule-forming bacteria (Lewis and McCoy, 77). The latter work gave unsatisfactory results, probably due to faulty technique. Riker and Berge have suggested the use of the method in studies of the crown gall organisms (99). It is to be hoped that, with the method itself firmly established, its application will be extended to other fields.

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# THE BOTANICAL REVIEW

VOL. II

SEPTEMBER, 1936

No. 9

## PLANT TUMORS AND THEIR RELATION TO CANCER

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Ewing (15) in his classical treatise, "Neoplastic Diseases," begins with the simple, though significant statement, "The ancients knew cancer well." Today, it may be said, so far as the etiology of human cancer is concerned, we are no further advanced than the ancients. This lack of knowledge, concerning the causative forces that induce cancer in man and animals, has made cancer a tempting field for the investigator in medicine, biology, chemistry and physics. Not only have the human aspects of the disease been studied, but animals and more recently plants have been made the subjects of experimental study with the purpose of bringing light to this obscure disease.

Many hypotheses have been evolved. In the early part of the 19th century it was believed that cancer was caused by drinking water of certain wells or streams. Such misconceptions as 'cancer districts' and 'cancer houses' were prevalent. Perhaps no explanation of the cause of this disease has had greater vogue than the parasitic theory. This hypothesis is the oldest of explanations of the cause of cancer. Even today, a parasite in the form of a virus is considered the etiological factor in certain animal tumors. Toward the end of the 19th and at the beginning of the 20th century new discoveries in the relation of bacteria and other microscopic life to disease were made. A host of parasitic organisms was described by as many investigators. Protozoa, bacteria, slime molds, yeast, and bread molds were described as the specific agents in cancer. Each in turn has been found lacking in the all important function of stimulating localized areas of somatic cells to repeated multiplication.



None of the advocates of the parasitic theory of cancer ever received the attention that was paid the work of Smith (76, 77, 78) and his collaborators, Brown, Townsend and McCulloch. The discoveries of Smith and his associates aroused the interest of animal pathologists and of phytopathologists alike. Interest was aroused in spite of the fact that they isolated a bacterium that produced tumors only in plants. Smith and Townsend (78) isolated a bacterium which, when introduced into a wound in any part of the plant, produced an overgrowth, a plant tumor, known as crown-gall, the etiology of which had been unknown at that time. This bacterium, named *Bacterium tumefaciens*, now frequently referred to as *Pseudomonas tumefaciens* (S. & T.), was isolated from small nodules found on the stems of the Paris daisy (*Chrysanthemum frutescens*). Smith and his collaborators showed that when this parasite was introduced into a wound made with a sterile needle, the cells in the inoculated area divided until a new growth, a tumor, was formed in the tissue of the plant and on, or over, its surface. When the tissue of the tumor was plated, i.e., when slices of this tissue, especially prepared, were placed on sterile agar, the organism developed on the agar and was used to produce similar tumors in other plants. Smith and Townsend's experiments fulfilled Koch's postulates completely.

The crown-gall disease of plants can resemble only cancer of the skin and is best visualized as a warty, globular mass of tissue, the surface of which is studded with a large number of smaller spherical bodies partially imbedded in the underlying mass of tissue. In other cases, the gall is perfectly smooth with a thin layer of epidermis covering its outer surfaces and may grossly resemble a sarcoma of a long bone. As the disease appears in nature, it is characterized by a smooth swelling or by an irregular, warty mass of tissue found on the surface of the stem above the roots. This region of the plant is commonly referred to as the crown portion of the plant, hence the name, crown-gall.

Prior to Smith and Townsend's discovery, the crown-gall disease had been known for a long time. As it is well known, the grape vine has been the subject of great concern to the wine growers of Italy, Germany and France. Before the introduction of the American grape, the crown-gall disease was responsible for considerable losses to vineyards of Europe. In fact, the disease had been

known in Europe for more than 75 years. Because of its destructive activities in South America, South Africa and in the United States, crown-gall was recognized as a disease of economic importance. As early as 1892 Smith reported on galls formed on the peach.

Toumey (81), without knowing at that time the origin of the disease, described his observations of the behavior of the tumors on the almond tree. He noted that the galls were formed in the spring and died in the fall, or during the quiescent period of the tree's growth. He further noted that the margins of the gall, where the latter is in contact with the healthy host tissue, served as a source of further infection during the active period of the tree's growth. The successive destruction of portions of the stem ultimately weakened it, until it was broken by some mechanical agent, such as the wind.

Hedgecock (21) further characterized the disease from a study of crown-gall infected apple trees and grape vines. Hedgecock observed the general systemic effects produced on the host by this disease. Dwarfing of the stem and etiolation of the leaves and green parts of the plant were pointed out as the diagnostic traits of the disease. Experimentally, dwarfing is produced only when a tumor is induced by inoculations of the apical meristem of the plant. Small tumors may be formed without these effects if the inoculations are made on relatively old parts of the plant. These take a much longer time to develop.

In Europe, the bacteriological aspects of the disease were studied. Corvo, Cuboni, Cavara, Scalia, Brizi and others attempted bacterial isolation from galls on the grape vine, rose, poplar and other plants. Cavara (12) isolated an organism from the grape vine crown-gall which may have been of a species related or identical with *B. tumefaciens*. However, he failed to produce convincing evidence of the tumor-inciting property of his organism, although his organism produced tumors experimentally.

To the animal pathologist, the discovery of a parasitic organism that produced cell proliferations resulting in irregular, globular masses of new tissue on the surface of the host appeared significant. It must be remembered that at this time, no animal tumor, either malignant or benign, could be produced experimentally. Spontaneous animal tumors were propagated with difficulty by

transplanting or grafting, as shown first by Loeb (50, 51) and Jensen (27). Rous' work on chicken sarcoma appeared in 1910 and gave further impetus to the parasitic theory of cancer. Rous (68, 69) showed that these malignant tumors in chickens could be propagated by means of cell-free filtrates or powdered desiccated tumor.

The plant tumors, nevertheless, were not readily accepted, if at all, by the pathologists, clinicians and experimenters to be cancer. Smith attacked the objections raised by the animal pathologist in a series of papers purporting to establish similarities between cancer of the animal and the crown-gall disease of the plant. Smith (70, 71, 72, 73) contended that cancer is a parasitic disease and it remained for the animal pathologist to discover the causative agent. Many animal pathologists ventured into the botanical field to test out Smith's contention that crown-gall is cancer and that human cancer is produced by a parasite.

Jensen was among the first (28, 29) to undertake an intensive study of the crown-gall. The ingenuity Jensen displayed in his studies of animal tumor grafts was directed to grafting induced tumors of the yellow beet on the garden red beet. Galls which he had produced on the red variety were grafted on the yellow beet. On the basis of these observations, Jensen concurred, in the main, that crown-gall resembles cancer. However, Levine (39) pointed out that while the grafts of the crown-gall of the red beet grow in a small percentage of cases on the yellow beet, the introduction of the parasite present in the inoculum produces a crown-gall of the host whether the transplant succeeds in growing on the host or not. Blumenthal and Hirschfeld (6, 7) studied the crown-gall disease and concluded that crown-gall was not identical with cancer although somewhat later Blumenthal, Auler, and Meyer (8) isolated a bacterium from human cancer which they contended produced cancer of rats and mice. Kaufman (30, 31) described a bacterial parasite like the organism of Blumenthal, Auler, and Meyer in cancer of the mouse. Robinson (64) believed these organisms isolated from cancer were related to *B. tumefaciens*.

Many bacterial parasites have been found associated with cancer but in no case has the investigator been able to establish the organism as the etiological agent. Bacteria as secondary invaders in cancer are well known. Levin and Levine (36) in a study of the

analogies between the crown-gall and human cancer concluded that the reaction tissue in crown-gall may be best compared with the inflammatory processes in infectious diseases of animals. Devoid of cell types found in animals, the plant is capable of reproducing only the cell that is injured, so forming a protective mechanism comparable in animals to scar tissue or granuloma. It has been shown by Levin and Levine that under conditions still unknown, this reaction tissue may become extensive and invade the normal tissue of the host, producing a destructive new growth. Under these conditions, the crown-gall is comparable to malignant cancer of animals and man.

#### TUMOR EMBOLI, METASTASES; TUMOR STRANDS

Smith (75) explored the cancer pathology literature to discover possible similarities that may exist between this plant disease and the malignant growths of man. For the purpose of this review, it will be unnecessary to dwell on the morphological structure or the physiological behavior of cancer to understand the difficulties that Smith encountered. Perhaps one instance of biological importance will suffice here: an essential characteristic of the malignant animal tumor is the production of similar growths, metastases, in parts of the body distant from the seat of the primary neoplasm. Metastases, or the dissemination of crown-gall by emboli to other parts of the plant, do not occur because of the structural characteristics of the plant. The movement of the fluids, blood, and lymph in the animal and the nature of the animal tissue favor, according to the accepted views of the present day, the separation of small clusters of cells from the main tumor and their transportation to distant parts of the body. Here the cells become lodged and proceed to grow, forming a secondary growth. This new growth consists of types of cells identical with those of the primary neoplasms. The plant fluid, sap, most likely passes through cell walls and cell pores, but particles as large as cells cannot pass through these walls even if they were separated from the primary crown-gall. The analogy between the neoplasms of man and animals and the crown-gall disease of plants does not hold in this important and fundamental point.

The layman's conception that malignant growths are characterized by the development of 'roots' has good foundation in the

studies of Handley (20) and others. Malignant tissues form strands of cancer cells from the primary growth along lymph channels until a favorable location is reached where the growth takes on a nodular form. Smith, Brown and Townsend contended that crown-gall produces strands of crown-gall tissue so that a new growth is formed from one already present. Robinson and Walkden (65), in their study of experimentally produced crown-galls, contend that the secondary tumors are formed when rudimentary wounded structures, subsequently separated by growth, are infected. Robinson and Walkden claim to have observed the bacteria in a jelly-like mass extending from the primary tumor to the secondary growths.

Riker (61) made a study of the secondary overgrowths on a number of plants. He contends that secondary growths are never formed except when the inoculations are made in rapidly elongating regions (embryonic structures) close behind the condensed buds. The mechanism of the tumor formation is explained on the assumption that the injury releases fluids which find their way in the intercellular spaces and bacteria, reaching these spaces, migrate in the medium and so induce secondary tumors. Riker states, however, that secondary galls and tumor strands are not really secondary at all from the standpoint of invasion. They are provided with bacteria from the same inoculation that produces the primary gall.

Levine (38) studied the formation of the so-called secondary tumors in the petioles of *Ricinus*, where they occur frequently. It was shown that when the apical bud of *Ricinus* was inoculated with *B. tumefaciens* the young leaves emerging from the bud may be so affected, that at maturity a gall is formed at the internode and base of the petiole, and one at the blade end of the petiole. Microscopic preparations of such petioles failed to reveal any connecting tumor strands. One must infer that the two areas were inoculated at the same time, but that development of the non-inoculated tissue separated the two inoculated parts. The tumor on the older part which becomes visible first has been referred to as the primary growth, while the younger gall which becomes visible later has been referred to as the secondary tumor. Levine was unable to verify the presence of bacteria in the deeply staining intercellular spaces of the plants he studied. Yet he is in accord with Riker in the

belief that the inoculation that produces the primary overgrowth is also responsible for the secondary gall.

It appears quite evident that metastases in crown-gall do not occur and that the so-called secondary tumors are produced by the same inoculation that causes the primary growth. There is no tumor cell continuity between primary and secondary tumor overgrowths in plants. The terms primary and secondary tumors in crown-gall represent a temporal relationship in the development of two galls arising from simultaneous inoculations of embryonic tissues. In cancer it is believed that the secondary growths arise directly from the primary growth, either by emboli or strands.

#### LOCATION OF BACTERIA IN THE HOST

The location of the bacteria is a mooted question. Smith, Brown and McCulloch in 1912 contended that the bacteria attack the nucleus and are found in the cell. Robinson and Walkden believe the parasites move by growth of a jelly-like substance in which they are imbedded in the spaces between the cells or that they may be found on the surface of the gall. Riker (61), Hill (23), Hill, Brittingham, Gibbons and Watts (24), Berridge (5) and Banfield (3) have affirmed this opinion. Petri (58), Pinoy (59), Magrou (52) and Němec (57) contend that the crown-gall organism and its related forms are intracellular. Magrou stated that the bacteria are also found in the intercellular spaces but exert their influence on the host cells at some distance from them. It appears that further work is necessary to establish the location of *Bacterium tumefaciens* in the host.

Banfield's latest report (3) of the minute overgrowths that appear on the fruiting canes of the black raspberry, and some limited studies of crown-gall show the parasite to be intercellular. Banfield made a careful study of the mitochondria, plastids and fat bodies in the normal tissues of the root and apical meristematic tissues of the black raspberry and contrasted these with the cytoplasmic inclusions in the gall tissue to obviate possibilities of mistaking these bodies for bacteria. Banfield claims that the organism appears primarily in the intercellular spaces in the host. Nevertheless, the intracellular presence of this organism is also affirmed. Butler (11) pointed out that the cane-gall is morphologically unlike the crown-gall and arises from pericyclic fibers. Berridge (5),

on the basis of a study of the pH concentrations of the crown-gall tissue, contends that the pH concentration of the plant sap most favorable for the growth of *Bacterium tumefaciens* is at 5.2. This pH concentration characterizes the meristematic tissue where the crown-gall tissue arises. The bacterial zooglea in the interspaces and on the surface of the tumor also show an H-ion concentration of 5, and in other parts of the tissue a pH concentration of 5.8. Quirk and Fawcett (60), in studying the pH concentration in culture media, show that the range of best growth of the hop strain of *Bacterium tumefaciens* in a 1% peptone beef infusion is at a pH of 5.7 to 9. It appears that Miss Berridge did not see bacteria in the tissues she studied but assumed their presence on the basis of staining reactions with methyl or diethyl red.

Brown and Quirk (10) contend that the pH of the freshly extracted juice of crown-gall tissue is always higher than that of the normal tissue. *Ricinus* with mature tumors showed an initial pH of 5.5. In rotted tumors the pH is 6.8, while in normal tissue the pH is 5.4 to 5.5. Brown and Quirk were concerned with the bacteriophage of *Bacterium tumefaciens* and did not have in mind the difference of the pH of young and old tissues. It may be that in the pea seedling, the meristematic tissue favoring the growth of *Bacterium tumefaciens* has a pH of 5.2, as pointed out by Berridge. Nevertheless, this would hardly explain the tumor formation of *Ricinus* and beet, for according to Brown and Quirk the normal juice of these plants shows a pH of 5.4 and 5.9, respectively.

It seems that the solution of this problem will be accomplished only when adequate photographs of the crown-gall tissue are made at a magnification sufficiently high to clearly show the rods or other forms of the parasite either in the intercellular spaces or in the protoplast of the cell. None of the reports so far presented shows the organism clearly. The photographs of Riker and Robinson and Walkden show masses of some foreign substance at the site of inoculation and in intercellular spaces removed from the point of implantation of the bacteria. Hill's drawings are well executed but fail to be convincing. Photographs of these bacteria made from smears have been published by Rosen (66), Magrou (52) and Levine (47).

It may be stated briefly that Smith (74) believed bacteria were not necessary to produce crown-galls but that the products of the



metabolism of the parasite were sufficient. The production of tumors in plants by chemical means has been the subject of considerable experimentation, but the reaction tissues so formed are small and do not reach the overgrowth stage, although intumescences and internal cell proliferations of a limited type have been reported (see Levine, 44-46, for further references to this question).

Kostoff (33, 34) calls attention to the spontaneous appearance of tumors in certain tobacco hybrids. These non-parasitic tumors are especially abundant on the roots and stems of hybrids resulting from crosses between *N. glauca*  $\times$  *N. Langsdorfii*. These species of tobacco have an unlike number of chromosomes. Kostoff contends that these tumors are not correlated with the chromosome number of the parents but are due to the mutual activity of the maternal and paternal contributors in the hybrid. Whitaker (85) reinvestigated these spontaneous growths on these hybrids and interprets the phenomena as due to a cytoplasmic disturbance occasioned by the introduction of the chromosome complement of *N. Langsdorfii* into the cytoplasm of the *N. glauca* used as the female parent. In fact, Whitaker holds that the *alata* group (*N. alata*, *N. Langsdorfii* and *N. Sanderæ*) produce tumor-bearing hybrids when *N. glauca* or *N. paniculata* are used as the female parent. When the *alata* group is used as the female parent the hybrids do not produce tumors. Crosses between members of the *alata* group or between *N. paniculata* and *N. glauca* never or rarely produce tumors, according to Kostoff.

Whitaker's interpretation of this tumor phenomenon is more specific and suggests the possibility of further studies to determine the origin of these interesting overgrowths. Kostoff's (33) precipitation tests to determine the tumor-producing activity of an agent were made on the *Ricinus* stem. Tests on this plant are not sufficiently critical, for the tissue in the lumen of the stem proliferates too readily (Levine, 46) after the introduction of any one of a host of substances.

#### CYTOLOGY OF CROWN-GALL TISSUE

Another aspect of the problem which widens the gap of analogy between cancer of animals and crown-gall of plants is found in the cytological phenomena of these two classes of overgrowths. It has been known that cells in human and animal cancer tissues are ab-



normal in their behavior. The earliest students of the cytology of cancer, Virchow (83), Arnold (2), Martin (53), Cornil (13) and Klebs (32), found atypical, aberrant cell structures. From 1890 to the present day the cytology of animal and human cancer has been studied diligently with the purpose of throwing light on the question of the etiology of this disease. It was hoped that some structure or behavior of the cancer cell would be discovered so as to serve as a means of making an early diagnosis of the disease in order to bring the known therapeutic measures to the relief of the afflicted.

Von Hanseman (84), V. Müller (55), Stroebe (80), Galeotti (18), Trambusti (82), Krompecher (35), Nedjelsky (56), and a number of other investigators described atypical asymmetrical divisions, monaster and multipolar spindles, and amitotic nuclear divisions in cancer cells. Cells with a larger number of chromosomes than normal for the species were considered by von Hanseman and others to be due to an unequal distribution of the chromosomes in nuclear division. On the basis of these phenomena various theories as to the cause of cancer have been evolved. Farmer, Moore and Walker (16), Aichel (1), Deton (14) and others believed cancer was due to fusion of somatic cells. Boveri (9) explained the cause of cancer on the basis of altered chromosomal behavior. Ross (67), Baur (4), Whitman (86), and others thought cancer a somatic mutation. Lewis (49) contends that the cancer cell is an altered one and capable of perpetuating itself. Malignancy is due to cytoplasmic alteration rather than chromosomal or gene changes. Smith, Brown, and McCulloch (77) studied the cell type and nuclear behavior in the plant overgrowths. Smith and his associates were impressed with the importance of bacteria as an etiological factor in cancer and contended that the foreign body giant cell and tumor giant cells are not very different, except that those that are induced by bacteria are malignant, while those that are activated by non-living granules are harmless. While the origin of giant cells of cancer is not clear, these two types of cells are distinctly separable on a cytological basis (Levine, 42). Without having completed their work on this phase of their crown-gall studies, Smith and his collaborators noted clearly that most nuclear divisions in crown-gall tissue were of the mitotic type. Their figures, however, stressed such abnormalities in nuclear divi-

sion as one frequently finds in studying neoplastic tissues of animals. It appears evident that the intention was to show closer relationship between cancer and crown-gall on a cytological basis.

Comparative cytological studies of human cancer and crown-gall tissues were made in 1925 (Levine, 37). It was shown that in cancer of the human lip, normal cells are found which divide mitotically. It was pointed out that a number of phenomena, such as aberrant mitotic divisions, were apparently due to failure to study serial sections of the material. Cells with multipolar spindles with many chromosomes were due mainly to the division of the nuclei of the various giant cell types. Asymmetrical distribution of the chromosomes on the spindle, which were stressed by the early workers, were shown to be due most frequently to the failure to observe two or more serial sections of the same cell. The cytological study of crown-gall tissue showed no aberrant nuclear behavior, no lobulate nuclei, no multipolar spindles and no amitotic divisions. It was noted that binucleate cells and multinucleate cells are common. The striking cytological difference between the crown-gall and cancer cells is the bizarre nuclear division phenomena in the latter, occurring in the giant tumor cells which were described as uninucleate or multinucleate cells.

Winge (87) studied the chromosome number in the sugar beet crown-gall and pointed out that cells in the tumor tissue have twice the number of the chromosomes normally found in the somatic cells of the beet. He contended that crown-gall cells have the increased number of chromosomes and that the normal somatic number of chromosomes found in some cells of crown-gall is due to a reduction of the larger number. The presence of these increased chromosome numbers in the tumor cells of the beet was verified (40, 41) and extended to crown-gall on the tobacco. The chromosome number in the cells of the cancer tissue of man, rat, mouse and bird was counted. A number of animal pathologists, Heiberg and Kemp (22), Lewis and Lockwood (48), Hirschfeld and Klee-Rawidowicz (25, 26), Goldschmidt and Fischer (19), and Levine (42), reported the chromosome numbers in animal tumors studied *in vitro* and by the paraffin method.

The results of the various studies made on the chromosome number in the animal cancer are essentially in accord. It was pointed out (42) that while the chromosome number in the animal

cancer tissue, including man, occurs generally in multiples of the known haploid number, there are variations from this: e.g., if we accept von Winiwater and Ogumas' (88) count in the somatic cells in man as 47-48, cells in the cancer of man show varying numbers: 26, 27, 48 and 67. We also find 96 (a tetraploid number), 136, 200 (close to the octoploid number 196), 300 and more chromosomes. In these latter cases Levine believes that the chromosomes divide without the intervention of spindle-fibers or the mechanism found in normal cells in division. In beet or tobacco crown-gall cells there is a distinct regularity in the number of chromosomes, e.g., in *N. glutinosa* the number of chromosomes in the normal cells is 24, while in the cells of crown-gall tissue we find 24, 48, 96 and possibly a larger number, a definite multiple of the haploid number. This may be explained on the basis of nuclear fusion in the absence of cell division.

Cytologically, the known overgrowths of plants present normal mitoses of cells with simple or fused nuclei. The spontaneous cancer of man or the tumors of the rat, mouse, or fowl, or the well known experimental strains of animal tumors that are propagated by grafting show aberrant mitosis and multipolar spindles. The resting cell in cancer may be normal in appearance and, at the present time, is considered physiologically normal, although Lewis (49) believes some cancer cells to be sick and the illness to be lodged in the cytoplasm. The other types of cells in cancer tissue are the tumor giant cells. These cells are either uninucleate with a uniform nuclear membrane or they may have an irregular boundary forming lobes or buds. Other types of giant cells are multinucleate with two or more nuclei or masses of fused nuclei. When the uninucleate cell undergoes division a single spindle is formed. When the other types of nuclei undergo mitotic division bizarre nuclear phenomena are observed. Yet tripolar and quadripolar divisions frequently occur in uninucleate cells. These types of divisions have not been observed in crown-gall tissue. Gaines and Aase (17) in the hybrid wheat and recently Emmy Stein (79) in progeny of irradiated *Antirrhinum* plants have shown giant cells, coalesced pollen mother cells, with atypical nuclear division, suggestive of animal tumor giant cells.

Riker's (62) studies on the cytology of crown-gall called attention to the cell inclusions which are commonly observed in sections

of old galls. These globular bodies vary in size but appear frequently of the same size in a given crown-gall cell. These bodies are conceded by Riker and Berge (63) to be of tannin, as pointed out by Milovidov (54). Milovidov studied the chondriosomes in the cell of *Pelargonium zonali* crown-gall and concluded that they do not differ from the normal chondriosomes as seen in the cotyledon of *Vicia faba*. The chondriosomes assume a typical form, stain typically and build starch. Crystals of calcium oxylate are also present. These substances are frequently found in crown-gall of the tomato, geranium, etc., as well as in the normal tissue.

Banfield (3), in his cytological studies on the cane-gall, came to a similar conclusion. The mass of gall tissue is initially composed of cells in every respect comparable with analogous cells of the meristematic region of the plants unaffected by cane-gall. Mucilage cells which constitute a conspicuous part of the histological structure of the cacti are absent in crown-galls experimentally produced in these plants (43-45).

In the non-parasitic tumors of the hybrid tobacco, Kostoff (33) reports cells with 21 chromosomes. This number is to be expected since the maternal element furnished 12 chromosomes and the male supplied 9 chromosomes. However, Kostoff (34) finds some cells in these tumors with 28, 30 and 42 chromosomes. The hybrid of *N. glauca*  $\times$  *N. Langsdorfii* when crossed back with *N. Langsdorfii* produced fertile plants with tumors. The cells of these tumors show as many as 48 or more chromosomes. Whitaker (85) found no cytological abnormalities in the tumor cells of the hybrid (*N. glauca*  $\times$  *N. Langsdorfii*). Chromosomes counted in over 40 cells showed no polyploidy. The mitoses were regular with little lagging or irregularity in chromosome behavior. Binucleate cells were not common. This apparent discrepancy may be attributed to variations in the tumor.

The differences pointed out above between crown-gall and cancer do not detract from the importance of Smith, Brown, Townsend, and McCulloch's discoveries. Crown-gall has at least one vital fundamental point in common with cancer, and that lies in the fact that crown-gall is a neoplastic disease consisting of a localized area of proliferating cells. While the proliferations are limited, i.e., because the gall ages, the botanist has the advantage in having an organism which, when injected into most plants, pro-

duces local cell growth and division. Crown-gall tissue represents essentially a fundamental tissue and, because of the simplicity of its host, is excellent material for the study of such problems as growth and reproduction and may thus throw light on the solution of the cancer problem.

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## THE OEDOGONIALES\*

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## INTRODUCTION

In the whole range of Chlorophycean genera there is perhaps no other order as distinctly separate and as unlikely to be mistaken for other algae as the Oedogoniales. This is especially true when reproductive structures are present. Even an amateur quickly learns to recognize without fail an *Oedogonium*, a *Bulbochaete*, or an *Oedocladium* by means of vegetative characteristics alone. The most outstanding features of the group, merely to mention them for the present, are the curiously rigid vegetative cells often with apical caps, the reticulate chloroplasts, the holdfast cells, the large zoospores with a crown of cilia at the anterior end, and, of course, the prominent oogonia and antheridia.

The single family, Oedogoniaceae, was established by De Bary (4) in 1854 and now includes three genera: *Bulbochaete* C. A. Agardh (2), *Oedogonium* Link (12), and *Oedocladium* Stahl (20). The systematics of the group, brought up to date in 1930 by Tiffany (24), were first put into an orderly fashion in a splendid monograph by Hirn (9). Unusual interest in the Oedogoniales, both in this country and abroad, has been manifest during the last few decades. Numerous papers have appeared describing new species, extending the geographic range and discussing various phases of reproductive and physiological activities. This report will endeavor to correlate some of these more recent investigations with our previous knowledge of the group.

The following summary table gives the present numerical status of the three genera. Incompletely described or doubtful species are not included.

| Genus                    | Species | Varieties | Forms |
|--------------------------|---------|-----------|-------|
| <i>Oedogonium</i> .....  | 296     | 49        | 18    |
| <i>Bulbochaete</i> ..... | 66      | 8         | 5     |
| <i>Oedocladium</i> ..... | 6       | 0         | 0     |

\* Papers from the Department of Botany, The Ohio State University, No. 370.

## DISTRIBUTION AND PERIODICITY

That many species of *Oedogonium* and *Bulbochaete* are widely distributed in fresh waters throughout the world is becoming increasingly evident with every algological survey of new regions. In fact, "geographical distribution" of many of these species means little more than the location of phycologists, past and present, who have interested themselves in the algae about them. Absence of precise structural limitations sometimes makes species differentiation hazardous, but these difficulties are just as likely to be met in collections from a single location as from widely separated habitats. In other words, speaking generally for the temperate and tropical realms, one is driven to the conclusion that the distribution of the species is dependent upon something much more basic than physical divisions or continental areas.

If we are able to put together the scattered data resulting from analyses of local habitats over a rather wide area, we may secure some picture of the ecological and physiological situation underlying the distribution of the Oedogoniales. Bukatsch (3) finds the optimum temperature for photosynthesis in certain species of *Oedogonium* to lie between 25° and 30° C. This is of course subject to variation due to the depth of submergence of the plant and to the difference in available solar radiation. Many species of both *Oedogonium* and *Bulbochaete* in North America, however, grow luxuriantly in waters of a temperature as low as 18–20° C. A few species are able to maintain themselves at temperatures slightly above freezing and may survive after being frozen in ice for several weeks. In very shallow quiet inlets on the south shore of Lake Erie *Oe. landsboroughi* (Hass.) Wittr. and *B. varians* Wittr., as well as a few other of the larger species, are apparently uninjured after a few hours each day in light of high intensity and water up to 50° C. In midsummer shallow water may become deficient in CO<sub>2</sub>, and the algae may die of starvation rather than because of any harmful effects of high temperatures and intense light.

Sexual reproduction is more likely to occur in *Oedogonium* and *Bulbochaete* if the water is slightly alkaline: Mainx (14) found the optimum to be pH 7.2 to 8.2. *B. varians* var. *subsimpler* (Wittr.) Hirn regularly fruits in Ohio at a pH as high as 8.8. Ackley (1) records 17 species of fruiting *Bulbochaete* and

*Oedogonium* as occurring in acid bogs of Michigan with pH varying from 3.4 to 6.8. It is thus seen that rather large differences in hydrogen-ion concentration are tolerated at least by some species.

Phosphates and nitrates, and perhaps the salts of calcium and magnesium, are important in the vegetative growth of *Oedogonium*. Prairie ponds and pools of Illinois and cypress swamps of Florida are much richer in numbers of both individuals and species than the cattail marshes along Lake Erie or the waters of the Ohio shales. Taft (22) finds that the Oedogoniales flourish in Oklahoma in both shale and sandstone areas, and McInteer (13) associates best growth of these filamentous algae with the fertile Bluegrass region of Kentucky.

*Oe. reinschii* Roy, long known vegetatively from northern latitudes of the United States and Europe, apparently maintains itself in such sections wholly asexually. Oogonial and antheridial filaments have been collected solely from southern Florida and only in the month of May.

Although an attempt to correlate abundance of *Oedogonium* and *Bulbochaete* with ecological associations and formations of land plants is somewhat hazardous, the following facts are significant, provided it is kept in mind that purely local data considered alone may materially alter any general conclusion. In America the waters most productive of the Oedogoniales, excluding *Oedocladium*, lie in the tall-grass prairies, the eastern deciduous forest, and that part of the southeastern conifer forest in the Mississippi Valley. The subtropical and tropical forest areas and grasslands of Florida, Brazil and similar regions are almost equally rich in species. The coastal plain perhaps ranks fourth. Far below these regions in productiveness are the short-grass plains, the semi-deserts, the western conifer forests, and, with few exceptions, the northeastern evergreen forest.

In general, then, the criteria for the growth and maintenance of many of the Oedogoniales appear to be quite simple: relatively quiet water, including ponds and pools, shallow parts of lakes, oxbows, and sluggish streams provided with the necessary mineral salts; absence of shade; mud rather than sand or easily movable bottom; a pH on the alkaline side; and a growing season of sufficient length to allow for completion of vegetative and repro-

ductive cycles. The nearly world-wide distribution of some species may be accounted for by the almost universal presence of these ecological conditions in bodies of fresh water. The fact that several species of *Oedogonium* are found in practically every country on the globe indicates that the genus is geologically old enough to allow for such distribution by natural means (e.g., birds, air currents, etc.) or that identical evolution occurred in similar habitats in many quarters.

The species of *Oedocladium*, on the other hand, perhaps because of our limited knowledge of the genus, seem to contradict the generality of distribution just enunciated. There are now known six species of *Oedocladium* (Tiffany, 25): one each from Germany, Massachusetts, Virginia, New Jersey, Florida, and Puerto Rico. It is interesting to note, in passing, that the first species on which the genus was established was found in Germany in 1891 and has not been seen since, and that the other five species occur in the eastern seaboard of the United States. Only one of the species of *Oedocladium* is aquatic, the remainder being terrestrial and growing on wet mud or sandy loam.

Hirn (9) reports three species of *Oedogonium* and one of *Bulbochaete* from brackish waters of Europe. Fritsch (6) collected vegetative filaments of the former genus as a part of the red and yellow snow of the Orkneys. Both genera are represented vegetatively at considerable elevations in alpine regions. The ratio of species found in such habitats to the total number in the family is about on the order of 1:50.

The relative importance of habitats for the growth of *Oedogonium* and *Bulbochaete* has been investigated by Tiffany and Transeau (26) for the north central States of America. Out of a total of about 1200 records of collections over a period of some twenty years they find: 54% of the species in permanent ponds, 23% in lakes, 15% in temporary ponds, 6% in streams, and 2% in stream oxbows. These percentages are based upon exact determination which necessitates the presence of fruiting material; otherwise, identification of species is not possible. The still-too-numerous pseudoscientists to whom holdfast cells in both *Oedogonium* and *Bulbochaete* must be adaptations for a life in running water will find it a bit difficult to account for the scarcity of these plants in streams.

Species of *Oedogonium* and *Bulbochaete* in many places fruit for the most part at rather definite seasons of the year. Usually the smaller the alga, the shorter the vegetative phase and the earlier the time of sexual reproduction; conversely, the larger the alga, the longer the vegetative period and the later the production of sperms and eggs. There are, however, some notable exceptions. In the north central United States maximal sexual reproduction is reached in May-July with a second lesser maximum in October. The latter is quite likely the development of a second generation.

In tropical and subtropical countries such definite periodicities do not obtain. Collections from Florida, some parts of the Coastal Plain, Puerto Rico, and Brazil appear to indicate that maximum fruiting follows the rainy seasons. This coincides with Transeau's (27) general deduction from data gathered in southern Illinois that maximal fruiting of algae occurs not in low-water periods when the ponds are drying up but rather in high-water periods. The presence of 30 species of *Oedogonium* and *Bulbochaete* in a single collection from a cypress swamp pool near Arcadia, Florida, in 1931 may be accounted for by the simultaneous germination of oospores of varying lengths of dormancy, as will be noted below.

A clear picture of periodicity in *Bulbochaete* and *Oedogonium* is made difficult by two other factors, at present practically unstudied: (a) the length of the dormant period in the oospore and (b) the persistence of vegetative material perennially or through zoospore production only. It is definitely known that while most oospores in the north central United States are dormant for the winter following their formation, some germinate in the same autumn and a few others lie in the mud at the bottom of ponds for several years. It seems quite probable that extreme desiccation due to the drying up of the water may be endured for considerable lengths of time. There is evidence for the reappearance of *Oedogonium* "waves" every three, four, five or even eight years. Mainx (14) finds that a rest period of a year is necessary before oospores of *Oe. gracilius* (Wittr.) Tiffany will germinate.

A few species, like *Oe. grande* Ktz., have been collected in fruit from ponds of Illinois every month in the year, although the records for December and January are few. In cool streams, in alpine regions, and in subarctic waters *Oedogonium* may survive vegetatively from year to year. Many species exist perennially in

a vegetative state although fruiting at rather definite periods. Such ubiquity of spore production as illustrated by *Oe. grande* is rare among the filamentous algae.

Some interesting daily periodicities in the production of sperms and subsequent fertilization of the egg have been observed by Spessard (19) (*cf. post*). Zoospores apparently may be produced from vegetative cells nearly any time up to the initiation of sexual reproduction. Changes of temperature of medium, transfer from distilled water to a nutrient solution or the reverse, exposure to light after storage in the dark are some of the methods in use in the production of zoospores at will. Gussewa (8) maintains that zoospore production is inhibited by too low concentrations of ferric salts and conditioned by the presence of a certain amount of free CO<sub>2</sub> in the water. In nature zoospores are not formed in running water so abundantly as in quiet water.

#### CELL STRUCTURE AND DIVISION

The vegetative filaments of *Oedogonium* are unbranched, attached when young and in many cases for some time thereafter, and composed in most species of cylindrical cells. A number of species, however, exhibit variations—usually constant, sometimes not—from the cylindrical shape of cell, such as capitellate, undulate, nodulose, ellipsoid, or even apparently sub-hexagonal. In *Bulbochaete* the filaments are unilaterally branched and made up of cells usually broader at the upper end and bearing long, basally bulbous setae. The species of *Oedocladium* are also branched, but there is a differentiation into erect and creeping branches with chloroplasts and into rhizoidal branches of long slender cells devoid of chloroplasts. In the single aquatic species of *Oedocladium*, however, both kinds of branches are green. The cells of *Oedocladium* uniformly lack setae.

Attachment to various substrata, including macrophytes, algae, and even other species of the Oedogoniaceae, is made by a holdfast cell in both *Bulbochaete* and *Oedogonium*. *Oedocladium*, on the other hand, is without a cell of attachment. Even when free-floating, however, there is no difficulty in ascertaining the apical and basal ends of the filaments in all three genera: definite polarity is maintained throughout the life of the plant.

The resting nucleus of the vegetative cell is large, usually circular and flattened in appearance, and generally lies just within the chloroplast midway between the ends of the cell. It has a distinct nuclear membrane, an abundance of chromatin at the nodes of the network just within the membrane, and one or more nucleoli (15). The chloroplast is elaborately reticulate with the strands of the reticulum either broad or narrow and usually parallel to the long axis of the cell. The numerous pyrenoids occur at the intersections of the reticulum and are surrounded by sheaths of starch plates. The accumulation of plates of starch elsewhere in the reticulum may entirely obscure the usual appearance of the chloroplast.

The cell walls of the Oedogoniales are rigid and usually devoid of conspicuous mucilage, except in some species of *Bulbochaete*. According to van Wisselingh (29), the cell wall of *Oedogonium* consists of an inner cellulose membrane with a peripheral investment of perhaps amyloid. Wurdack (30) finds the cellulose inner layer to be bound externally by a zone of pectose which is covered in turn by chitin. Recent unpublished work seems to confirm both the cellulose and the pectic compounds, but throws some doubt upon the certain identity of the chitin. The outermost layer is undoubtedly not cellulose, but its exact chemical nature will have to await a more refined microchemical test than now in use. The composition of the cell walls of both *Oedocladium* and *Bulbochaete* is apparently similar to that of *Oedogonium*. The holdfast cells are generally devoid of this peripheral, chitin-like substance (23).

In the past three quarters of a century cell division in *Oedogonium* has been investigated by various workers. Perhaps the best accounts in more recent years are those of van Wisselingh (29), Tuttle (28), Kretschmer (11) and Ohashi (15). The nucleus moves distally from its mid-position between the end septa and comes to rest at a position about one-third the distance from the upper end of the cell. After enlarging and changing shape somewhat, it divides mitotically. At prophase most investigators report the appearance of a ring, which Steinecke (21) believes to be hemicellulose, completely enveloping the inner face of the lateral wall just below the upper extremity of the cell.

Whitford\*, who has recently made microchemical analyses of all three genera, feels that the ring "is not an isolated mass of wall material formed at that particular spot, but is merely an inwardly bulging thickened portion of a complete new layer laid down inside the old wall and entirely inclosing the protoplast. Upon the stretching of the ring the distal cell (to be) is inclosed by a single wall layer, except under the cap, composed of the distended ring; the forthcoming proximal cell, however, is inclosed by two or more wall layers, except at the distal end". The protoplast may divide before the ring stretches, but the transverse wall is found only after the daughter cells cease elongation. The septum soon connects with the lateral walls, and separation into two cells is complete. After a number of divisions the distal cell is surmounted by a number of shallow telescoping (apical) caps, each representing a division. In the large species of *Oedogonium* and probably in most species of *Bulbochaete*, the lower part or sheath cell may also divide and after a number of divisions is inclosed, except at the top, by a corresponding number of layers. Whitford has found cellulose in the ring long before any evidence of stretching appears, but all tests for hemicellulose are negative.

Cell division is intercalary in *Oedogonium*, resulting in cells with varying numbers of apical caps; it takes place usually terminally in *Oedocladium*, and so the end cell is generally the only one with the caps; in many species of *Bulbochaete* it is strictly basal and thus rarely more than one apical cap to a cell. In *Oedocladium* the caps may be pushed aside or disappear entirely after some growth of the plant.

Early cytological investigation of cell division in *Oedogonium* did not, of course, include a study of chromosomes. More recently, however, van Wisselingh (29) reports 19 chromosomes in *Oe. cyathigerum* Wittr., Ohashi (15) counts 13 in *Oe. grande* Ktz., and Kretschmer (11) finds 15 in *Oe. pachyandrium* Wittr. It is of interest to record this uneven number of chromosomes and to speculate upon the genetic behavior at reduction division. Ohashi (15) notes in cell division in *Oe. grande* that the chromosomes at metaphase show great diversity in form and shape. The forms usually assumed are short rods, long rods, and loops.

\* Communication from Prof. L. A. Whitford, North Carolina State College, Raleigh.



Division of the cells of *Oedogonium* takes place rapidly if external conditions are favorable. Slight desiccation of young filaments followed by immersion seems to accelerate cell division. Freund (reported by Fritsch, 7) maintains that a deficiency of requisite salts tends to stop division, the cells lengthening and becoming filled with reserves. Heavy incrustation of the filaments with calcium carbonate slows the process of cell division but does not prevent its occurrence. The recently formed cells, in such cases, stand out prominently because of the entire absence of the peripheral deposit.

#### ZOOSPORE FORMATION AND GERMINATION

Multiciliate zoospores arise singly from vegetative cells of all three genera. The formation and liberation of the swimmers can readily be followed if plants, previous to sexual reproduction, are brought into a warm room and observed from time to time under a microscope, (*cf. ante*). The first noticeable change in the vegetative cell about to form a zoospore is a slight contraction of the protoplast and the appearance of a colorless area between the nucleus and the wall. Around the margin of the hyaline area appears a ring of blepharoplast granules, each perhaps giving rise to a flagellum (Smith, 1934). Kretschmer (11) describes two rings of basal granules forming flagella in zoospores, but Gussewa (8) reports only a single ring-shaped blepharoplast in *Oe. capillare* (L.) Ktz.

A circular rent appears near the upper extremity of the cell. Steinecke (21) reports the change of cellulose into amyloid at the place of rupture, just previous to the appearance of the aperture. The zoospore slowly emerges in an amoeboid fashion inclosed by a pectic sheath, the liberation requiring but a few minutes. Steinecke (21) assumes that rupture of the wall and subsequent emergence of the zoospores are due to the swelling of gelatinous substances secreted at both ends of the cell by the protoplast. The gelatinous vesicle at first enlarges, then rapidly disappears, after emergence of the zoospore. The spore moves sluggishly while the gelatinous material is dissolving, but becomes very active upon the formation of the crown of flagella around the clear anterior end, from granules as noted above.

The zoospores are spherical or ovoid or pyriform and quite green in color except for the colorless beak around the base of which the flagella are attached. Mainx (14) reports the uniform presence of an eyespot. Zoospores are usually active for an hour or more before coming to rest. Fritsch (5) observed the formation of further zoospores from the one-celled stage. Usually, however, the quiescent zoospore in *Oedogonium* forms attachment by the development of a holdfast cell, the complexity of which apparently depends upon the nature of the substratum. Subsequent division of the sporeling forms the filament characteristic of the genus to which it belongs.

In *Bulbochaete* the first division of the zoospore is of the simple type without formation of a ring. The upper part of the cell is separated from the lower by a circular rent, but no new membrane develops between the two parts. As the new wall grows upward, the separated part is pushed to one side as a lid. The upper new cell grows out into a long tubular bristle with a swollen bulbous base. Subsequent divisions occur with the regular ring formation, and each new cell is intercalated between the basal cell and the next one above. The upper cell of each filament is, therefore, the oldest one. A lateral protusion of each cell at its apex is cut off, developing into a hair. It is thus seen that the terminal cell bears two hairs, the other cells one each. Nearly every cell of *Bulbochaete* has two apical faces, one bearing a hair (or a cell of a side branch), the other the next upper cell of the main axis. Every cell of the principal axis of the filament, except the basal one, may cut off by an oblique division a lateral cell which becomes the basal cell for a side branch. By repeated divisions, as noted above, this secondary basal cell forms the branch.

The first cell formed in *Oedocladium* upon germination of the zoospore may develop variously: into a green filament without early branching or with a rhizodial cell as a branch or with a green cell as a branch. No holdfast cells are formed. The formation of a branch in *Oedocladium* occurs by development of a circular rent in the upper extremity of a vegetative cell, forming an upper short "lid" and a lower cylindrical piece. Through this rent the protoplasm of the forthcoming daughter branch cell with its initially thin wall protrudes. A new wall separating the branch cell from the cell of the main filament is then developed. This

wall is attached to the juncture of the upper extremity of the cylindrical piece of the primary cell and the lower end of the branch cell, on the one hand, and to the original transverse wall of the primary cell, on the other.

Asexual reproduction other than through zoospore formation is effected also through formation of akinetes, although they are relatively uncommon. In *Oedogonium* the akinetes occur in the ordinary filaments either singly or in chains of 10 to 40 in number (18). In terrestrial species of *Oedocladium* they develop similarly in the rhizoids and are frequently rich in reserve foods and nearly always red in color. Akinetes germinate directly into new plants. When filaments of *Oedogonium* are heavily incrustated with lime or infested with fungal parasites, single aplanospore-like structures appear as spheroid or ovoid bodies in the middle of the vegetative cell. The entire protoplast appears to take part in their formation. The fate of such a "resting spore" is unknown.

#### SEXUAL REPRODUCTION

Sexual reproduction is of an advanced oogamous type in all the Oedogoniales, and certain phases of it are so unique as to have developed a very specialized terminology. External conditions must be suitable (*cf. ante*), and chief among these is a hydrogen-ion concentration on the alkaline side, which Mainx (14) sets at a pH of 7.4 to 8.2 for most filaments. It should be kept in mind, however, that a certain development or maturation within the plant, the acquiring of a so-called sexual tonus, is probably more important than any special complex of environmental factors. As a matter of fact, when this "state" has been reached, it is practically impossible to prevent sexual reproduction in many species of *Oedogonium* without destroying the filaments. It is interesting, in passing, to recall that ponds of prairie areas produce fruiting material much more abundantly than those of many other regions generally poor in necessary mineral salts.

Perhaps the most unusual characteristic of the reproductive structures of the Oedogoniales is the dwarf male, a short filament epiphytic on or near the oogonium and usually composed of a holdfast cell and one or more antheridia. Species with such dwarf males are "nannandrous" and always dioecious. If there are no dwarf males, the plant is "macrandrous" and as such may be either

monoecious or dioecious. The dwarf males arise from the germination of androspores, which are zoospore- or gamete-like swimmers produced in short cells known as androsporangia. If the androsporangia are formed on the same filament as the oogonia, the species is "gynandrosporous"; if on separate threads, the plant is "idioandrosporous." It should be pointed out that a very few macrandrous species of *Oedogonium* are either dioecious or monoecious and that some of the nannandrous forms of both *Oedogonium* and *Bulbochaete* are either gynandrosporous or idioandrosporous.

Apparently any vegetative cell, except the basal one in *Bulbochaete* and *Oedogonium* and the rhizoidal cell in *Oedocladium*, may become an oogonium-mother cell. Just why relatively few cells of any given filament are differentiated into oogonia is not known, but it is perhaps associated with abundance of food reserves. In *Oedogonium* and *Oedocladium* a transverse division of a vegetative cell results in two segments. The upper one enlarges and becomes the oogonium; the lower segment or sheath-cell forms the supporting or suffultory cell. In *Oedogonium* the suffultory cell may undergo further segmentation, resulting in seriate oogonia. In some species the swollen nature of the suffultory cell is of diagnostic value. Ohashi (15) reports the absence of a supporting cell in *Oe. americanum* Transeau.

In *Bulbochaete* the oogonia are not seriate. An oogonium arises by a double division of a vegetative cell and so has two suffultory cells that may be of equal or unequal length. The relative lengths of the two supporting cells are fairly constant features for a given species in the genus. If the two divisions of the oogonium-mother cell are at right angles to the long axis of the vegetative cells, the oogonium is "erect"; if one division is oblique, giving one suffultory cell a five-sided, or five-angled, appearance (viewed in optical section), the oogonium is "patent." In members of the genus with globose (or nearly so) oogonia, the upper and not the lower suffultory cell is five-angled, while in those with more ellipsoid oogonia the reverse is true. In the latter case the upper cell is quite small, or may not be visible. A patent oogonium is always formed by a division of the basal cell of a side branch, with which may be associated a bulbous hair, an androsporangium, an antheridium, or rarely a vegetative cell. An erect oogonium on the other hand forms from a division of some vegetative cell other than

the basal one. In the globose forms of the genus, patent oogonia are common while erect oogonia occur only in a few species. Among the ellipsoid forms both kinds are found.

The oogonium is of various shapes and with few exceptions has a diameter greater than that of the vegetative cells. It contains a single egg having a centrally placed nucleus in early stages of development (15). As the oogonium nears maturity, a small pore forms in the wall by the appearance of soluble pectic material at the tip of a papillary projection; in some species a similarly formed transverse slit may separate the lower from the upper part of the oogonium, resulting in a lid. The position of the pore in the poriferous species and of the slit for the operculate species is quite constant for a given species and has real diagnostic value.

The antheridia of macrandrous species are flat, discoid cells developed by repeated transverse divisions of a vegetative cell. The first division is always near the upper part of the cell, and subsequent partition of the lower segment of the mother cell may result in a series of 2 to 45 antheridia in a single filament. The protoplast of the antheridia may upon maturity form a single sperm, but it usually forms two sperms, either by a transverse or a vertical division. Liberation of the sperm is practically identical with that of the zoospore described above.

Antheridia of nannandrous species are produced on dwarf males that result from germination of androspores. Flat discoid androsporangia, formed from vegetative cells in the same way as the antheridia of macrandrous species, produce each a single swimmer or androspore. Upon escape from the androsporangium in the manner of a sperm or zoospore, the androspore comes to rest, after about an hour, usually on the oogonium or suffultory cell. In some species of *Oedogonium* and rarely in *Bulbochaete* the androspore may become affixed to a vegetative cell considerably remote from the oogonium. Such cells are assumed to be potential oogonium-mother cells that never became morphologically differentiated. In some collections of *Oedogonium* from Florida the writer has observed a few germinated androspores on unrelated filaments of the same genus or even on cells of *Cladophora*. At any rate the androspore grows into a sporeling with a holdfast cell and one or more antheridia, the latter forming as in the macrandrous plants. In some cases the dwarf male may have a few vegetative cells

between the holdfast cell and the antheridia. Some nannandrous species, especially the smaller ones, have one-celled dwarf males, and in such cases the upper part of the holdfast cell acts as an antheridium. Each antheridium normally contains two sperms, formed by a horizontal division of the protoplast.

The dwarf males of the Oedogoniales have been the subject of much speculation by phycologists. Pascher (16) regards the species possessing dwarf males as the more primitive since three kinds of swarmers are really present: zoospores, androspores, and sperms. The intermediate androspores are zoospores that are incapable of complete vegetative development and so give rise to dwarf filaments producing male cells. Further evolution of the protoplast of the androsporangium made it possible to produce sperms directly. The macrandrous forms are thus to be regarded as the more highly specialized.

Most investigators incline to the view, however, that the macrandrous group gave rise to the nannandrous. It is common observation that many of the ordinary male filaments are frequently smaller than the female and that quite young dioecious plants may form antheridia. These two facts are used as evidence to support the claim that the dwarf males are but reduced antheridial filaments of dioecious macrandrous types. Schaffner (17) regards androspores as modified sperms that have retained some degree of sexuality; that is, they react sufficiently to the oogonium or suffultory cell to lodge there, but not sufficiently to fuse with the egg. An androspore may thus be regarded as a sperm that develops parthenogenetically into a dwarf male. The presence of dwarf males on cells of *Cladophora*, mentioned above, may be accounted for by the proximity of oogonial filaments of *Oedogonium* at the time of the attachment and germination of the androspores.

Fertilization in all three genera occurs after the sperm swims through the opening in the oogonial wall. According to Spessard (19), the sex organs of *Oe. kurzii* Zeller are mature in about 48 hours after their differentiation from ordinary cells, antheridia are formed about a day after the oogonia—thus seemingly insuring cross fertilization—and maximal sex organ production occurs about every fifteen days. The sperms of this same species are liberated throughout the period of sexual activity, but maximal emergence occurs between midnight and four A. M. A second

lesser wave may be seen from noon to four P. M. Fertilization in *Oe. kurzii* may take place within ten minutes after the discharge of the sperm. Passage of the male gamete through the conduit may require from a few seconds to four or five minutes, but the actual entry into the egg consumes only a half minute. The cilia of the sperm have long been regarded as relatively short structures, but Spessard reports those for *Oe. kurzii* to be much longer than the body of the gamete itself.

The sperm makes contact with the egg anterior end first in *Oe. kurzii*, which is contrary to the usual interpretation placed on the figure of Klebahn (10) for *Oe. boscii* (LeCl.) Wittr. The contents of the female gamete are pushed inward by the entering sperm and the membrane immediately "heals" over after the posterior end of the male cell has completed passage. Fusion of the two nuclei occurs without delay. The male nucleus is always the smaller and devoid of a nucleolus (15). The female nucleus before fertilization moves from its central position in the egg near the oogonial aperture. The fusion nucleus is somewhat globular in shape. Differentiation of the spore wall soon becomes noticeable, and the mature oospore has three (sometimes two or even four) membranes: the inner one nearly always smooth, the middle one often highly ornamented, and the outer layer sometimes ornamented, more often not. The ornamentations are quite constant in the oospore of a given species, and thus furnish excellent criteria for accurate identification.

The oospore of *Oe. gracilius* undergoes a rest period of a year before germination. The species becoming sexually mature in autumn, however, have grown from oospores whose dormant period was six months or even less. There is little doubt, on the other hand, that dormancy (*cf. ante*) may be a matter of several years in some species of *Oedogonium*. Mainx (14) observes that after the elapse of a year, 100 per cent germination of oospores may be obtained after 24 to 48 hours in ice-water and then transference to a nutrient solution. Normally, the oospore of *Oe. gracilius* produces 4 zoospores, two of which give rise to antheridial filaments and two to oogonial filaments. This may be the case in all dioecious plants of *Oedogonium*. Although unable to ascertain either the haploid or diploid number of chromosomes, Gussewa (8) gives evidence for reduction division at the time of germination of the



oospore in *Oe. capillare*. This confirms what has long been assumed, and it is quite probable that all Oedogoniales are haploid. Not all of the four nuclei formed in germination of the oospore may produce zoospores, and this accounts perhaps for the occasional observation of only one or two or three swarmers from a single zygote. It has been suspected that the oospore of some species may under certain conditions germinate directly into a filament, but this as yet lacks confirmation.

Another observation by Mainx (14) is unusual. In some cases a zygote gives rise to a single, large, presumably diploid zoospore which grows into a filament twice the normal size and always female. The oogonia of such plants are, however, flatter in appearance, and their formation in series suggests antheridia. Mainx regards the factor for femaleness as dominant, but the recessive factor for maleness may show itself in the "antheridial" features of the oogonia. The eggs produced from such oogonia may be fertilized by normal haploid sperms, but further development of the presumably triploid oospore is not known.

Parthenospores have been seen in *Oedogonium* from time to time, and Gussewa (8) records their formation in *Oe. capillare* from the entire contents of the vegetative cells. They develop thick walls and occupy all the space within the cells except the corners. They look much like oospores, but neither a pore nor a slit is in evidence in the mother-cell wall. Gussewa's statement that the parthenospores germinate immediately into new filaments is difficult to accept in view of the presence of such a thick investment.

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## GLOSSARY

akinetes: in the green algae, single vegetative cells developed into spore-like resting stages with much thicker walls and more abundant food reserves. See G. M. Smith: Fresh-water Algae of the U. S. They may separate from the thallus or germinate *in situ* (as in *Pithophora*).

aplanospores: non-motile spore-like cells developed from vegetative cells (singly or not) by the formation of a new wall around the protoplast distinct from the mother-cell wall; to be regarded as abortive zoospores in which the motile phase is omitted. Smith, *supra cit.* (p. 286). They may occur in both filamentous and unicellular algae, not necessarily detached, then.

blepharoplast: specialized protoplasm giving rise to the motile cilia of antherozoids.

hemicelluloses: carbohydrates which are polymers of pentose and hexose sugars; hydrolyzed by hot dilute acids and not colored blue by chlorzinc-iodide. Anderson & Jackson.

macrophyte: any macroscopic plant.

oogamous: having sexual reproduction by unlike gametes.

oogonium: a female sexual organ, often a spherical sac, containing, in the Oedogoniales, one egg.

oospore: the immediate product of fertilization in the algae under discussion.

pH: the symbol for the logarithm of the reciprocal of hydrogen-ion activity. Values indicate degrees of alkalinity or acidity; pH 7 is neutral, higher values more alkaline, lower values more acid.

pyrenoids: minute rounded granular colorless bodies embedded in the chromatophores of green algae and associated with starch formation.

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# THE BOTANICAL REVIEW

VOL. II

OCTOBER, 1936

No. 10

## THE CHEMISTRY AND PHYSIOLOGY OF THE PECTINS

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### INTRODUCTION

The pectins are a heterogeneous group of carbohydrate-like, colloidal substances of extremely wide distribution in plant cell walls. Their existence has been known for many years, in fact, since 1824, when Payen (56) and Braconnot (11) described "gelée végétale" and "le principe gélatineux des fruits," substances which we now realize must have been pectins. These two workers dealt only with pectins which they could obtain in solution outside of the plant. Fremy (27, 28) recognized, however, that an insoluble pectic substance is present in cell walls, and that from this the pectins of Payen and Braconnot might be derived by boiling with water or dilute acid. This precursor of soluble pectin he called "pectose" or "protopectin." Still a third type of pectic substance was recognized by Fremy. This was obtained by heating material containing protopectin with dilute alkali. It behaved very differently from "pectin" in that it was precipitated by calcium. In addition, its solutions were acid whereas those of pectin were nearly neutral. He called this third type of substance, therefore, "pectic acid."

We have, then, three general types of pectic compounds:

1. Protopectin, insoluble in water, and found only in tissues.
2. Pectin, soluble in water, and obtained from No. 1 by acid hydrolysis; not precipitated by calcium.
3. Pectic acid, obtained from No. 1 or No. 2 by alkaline hydrolysis; precipitated by calcium.

This is the historical conception of the pectic substances. Further, it is hardly profitable to go historically in the development of our present knowledge, and I shall, therefore, rather discuss the present status with only an occasional reference to the older works.

## PECTIC ACID

Let us first consider pectic acid. The major constituent of pectic acid is galacturonic acid, or, more properly, galacturonic acid anhydride (23). Various workers have obtained various percentages of galacturonic acid in pectic acid. It may vary from 65 per cent to 95 per cent of the total. This variation is independent of worker and method and depends upon the fact that different pectic acids do really contain different proportions of galacturonic acid. The balance is made up of two substances, galactose and arabinose. The ratio of galactose to arabinose may also vary. Nanji, Paton and Ling (52) found, for example, a 1:1 ratio in several samples. Sloep (59) and also Ehrlich (24) have found that some pectins can be practically free of galactose, with arabinose making up one-fifth, galacturonic acid four-fifths, of the total. Ehrlich has also shown that some pectic acid may be practically arabinose-free. It is, then, galacturonic acid which is the characteristic constituent of pectic acid. Arabinose, galactose, or both may be present in varying amounts, but their quantitative relationships possess a large degree of flexibility.

The constituent molecules of pectic acid are present in the anhydride form, (*i.e.*, as in polysaccharides) and can be set free from one another by hydrolysis. It is clear, therefore, that in pectic acid itself they are linked together in some manner. For reasons which will become apparent later, they are not linked through their carboxyl groups, but rather through the usual polysaccharide linkages. There have been various schemes proposed for the manner in which this takes place. Nanji, Paton, and Ling, have, for example, proposed a "basic unit" composed of one molecule each of galactose and arabinose, with four molecules of galacturonic acid, the whole joined in a ring. Such a basic unit would, however, hardly allow for the great variation in constitution of pectic acid. Ehrlich, by continued careful hydrolysis of pectic acid, was able to obtain (a) a galactose-galacturonic acid, (b) tetra-galacturonic acids, some of which were clearly not in ring form, some of which he believed to be in the form of rings of four molecules. Since hydrolysis, Ehrlich believed that *they* formed the "basic units" of the tetra-galacturonic acids were very stable and resistant to further pectic acid and that galactose and arabinose were attached in some unspecified but loose fashion to this nucleus.

Other schemes for the structure of pectic acid have been advanced. There are, however, facts which indicate that these schemes as well as those of Nanji, Paton, and Ling, and of Ehrlich are fundamentally in error. As mentioned above, even the variable composition of pectic acid argues against the existence of such a "basic unit."

In the first place, the units of pectic acid (and of its derivatives) which are dispersed in colloidal solution are greatly elongated. The colloidal micelles are 100 times or more longer than they are wide. This elongated structure of the micelle finds its expression, for example, in the fact that when pectic acid is dried under tension in the form of threads, the micelles show a good orientation in the direction of tension. Among the markedly elongated colloidal micelles with which we are thus far acquainted, the micellar anisotropy is due to a molecular anisotropy. The best studied examples of this kind are cellulose and xylan, in which glucose and xylose, respectively, are connected by oxygen "bridges" into long chains. Moreover, these two substances are related to one another in that cellulose may be converted to xylan by removal of the sixth carbon atom of each glucose molecule. These sixth carbon atoms, as may be seen in figure 1, play no part in the main chain, and are merely "side chains." In pectic acid, one has a substance made up of galactose, arabinose and galacturonic acid, which differ from one another only in the sixth carbon atom. It seems, therefore, most reasonable to suppose that pectic acid consists of long chains of galactose molecules (Fig. 2, A), in which the sixth chain carbon atoms are free; that many of these sixth carbon atoms are oxidized to carboxyl groups (galacturonic acid, Fig. 2, B), and that some of them may be removed completely (arabinose, Fig. 2, C). Variation of the composition of pectic acid is readily understood on the basis of this structure, as is also the fact that pectin in nature is generally accompanied by greater or smaller amounts of araban (24).

The chain structure of the pectins can be verified in different ways, the most important of which is the X-ray method so successfully applied by Sponsler (62) to cellulose. Meyer and Mark (48), and particularly Van Iterson (71), have shown with this method that the galacturonic acid residues of pectins actually are arranged in chains but much work remains to be done concerning the detail

of the structure. By the X-ray diffraction method it is possible to obtain information as to the structure of the chain but not as to its length. The viscosimetric method of Staudinger (63) enables one, however, to determine the latter quantity. Henglein and Schneider (36) have applied this method to the pectins (actually to the nitropectins for technical reasons) with great success and have shown that in soluble pectin 200 or more molecules are joined in each chain.

In nature the pectic substances are always found in the isotropic state, and even in the laboratory it is difficult to cause the chains to assume even a relatively regular orientation. This, Frey-Wyssling (30) suggests, may be due to a low order of symmetry of the pectin chains. We know that starch, which consists of glucose molecules in chains as does cellulose, crystallizes with much more difficulty than does the latter. This seems to be due partly

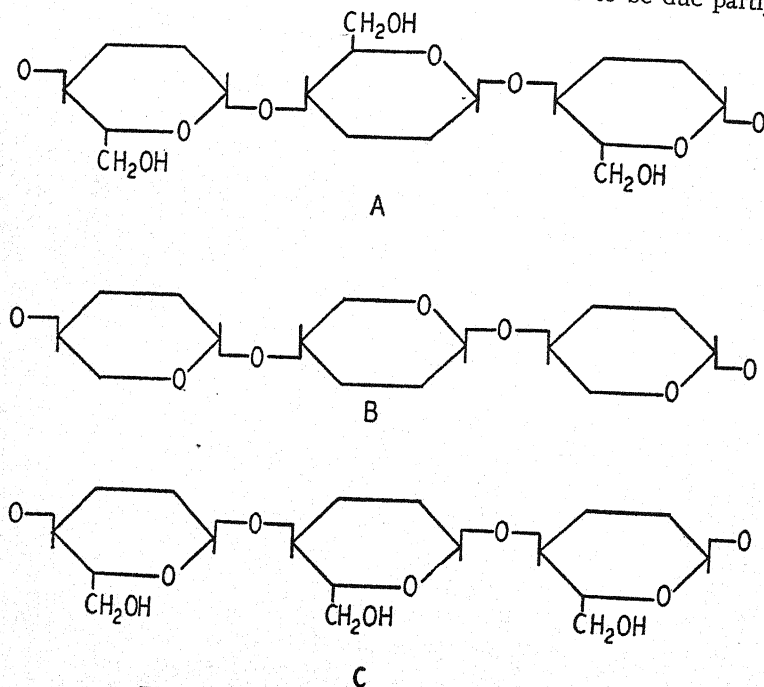


FIG. 1. Chain molecules of the glucose series:  
 A. Cellulose ( $\beta$  glucosidic linkages).  
 B. Xylan,  $\beta$  glucosidic linkages.  
 C. Starch,  $\alpha$  glucosidic linkages.

to the fact that in starch the molecules are bound by  $\alpha$ -glucosidal bridges whereas in cellulose they are bound by  $\beta$ -glucosidal bridges, as is shown in figure 1. By translation and rotation through 180 degrees each glucose molecule of cellulose can be superimposed upon the glucose molecule next ahead of it. In starch this is not the case, and this lower symmetry is apparently associated with a lower stability in the crystalline form. Frey-Wyssling, therefore, suggests that the chain of pectic acid, which also crystallizes with difficulty, possesses the lower grade of symmetry, as shown in figure 2.

Pectic acid does not occur as such in nature; the COOH groups are always combined in some manner or other. The combination of greatest interest is the methyl ester. All of the carboxyl groups may in theory be esterified with methyl alcohol. We would have then a pure *pectin*. In a few cases in the literature one finds pectins in which practically every carboxyl group is esterified (73, 65). In most cases, however, only a portion of the carboxyl groups are esterified and the remainder are free. It is also possible to saponify pectin by stages, and to obtain in this way a complete series of *pectinic acid*, partly methylated pectin derivatives ranging from pectin to pectic acid. We have, then, a series of compounds related to one another as follows:

|                |                       |                       |                         |
|----------------|-----------------------|-----------------------|-------------------------|
| pectin         | completely methylated | no free COOH groups   | } commercial<br>pectins |
| pectinic acids | partly methylated     | some free COOH groups |                         |
| pectic acid    | not methylated        | all COOH groups free  |                         |

As we shall see later, the differences in the properties of these compounds are related principally to the difference in number of free COOH groups.

The protopectin of the primary cell wall is not directly soluble in cold water alone. If, however, it is subjected to the mildest acid hydrolysis it may immediately be extracted as pectin or a highly methylated pectinic acid. Different reasons have been adduced to account for the insolubility of protopectin. Sucharipa (65) believed pectin to be a compound of pectin with cellulose. Sloep (59), however, and also Branfoot (12), have been unable to confirm his work and at present there is certainly no positive evidence that pectin is combined with cellulose. A molecular chain structure of the pectins would make the insolubility of protopectin much more easily understood. It has been shown for a large variety



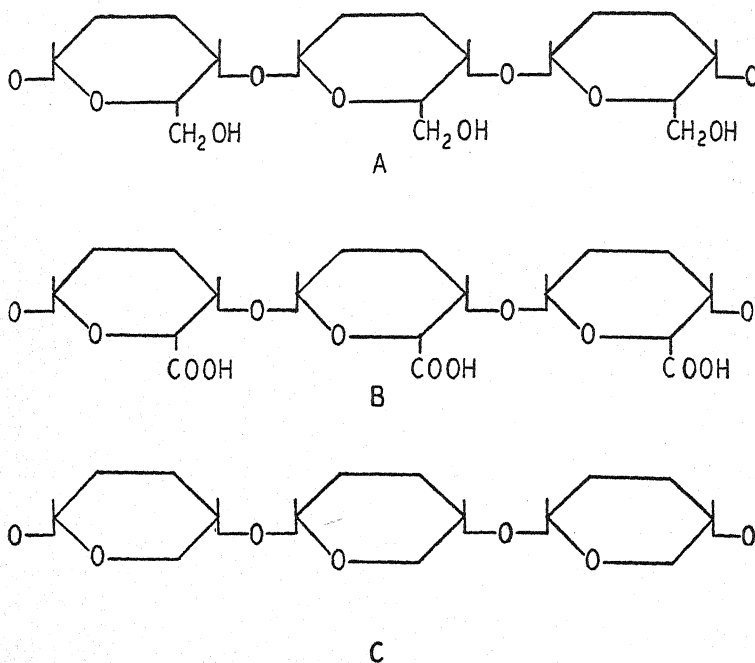


FIG. 2. Chain molecules of the galactose series:

- A. Galactan.
- B. Poly-galacturonic acid.
- C. Araban.

of such compounds that the longer the chain the more insoluble the substance (63), and that, in addition, the longer the chain the more easily it can be split by hydrolysis, *i.e.*, the longer the chain the greater the chance that one of the links can be broken. It would seem possible, therefore, that protopectin differs from pectin in that the former is composed of longer chains. If this is the case the relatively greater ease of hydrolysis of protopectin would be at once understandable.

Let us again consider pectic acid as made up of long chains of galacturonic acid with an occasional galactose and arabinose scattered along the chain. If one adds a salt with a polyvalent cation, say  $\text{CaCl}_2$ , to a sol of pectic acid, one should expect an increase of "effective electrostatic attraction" (14) between the chains, and, if this attraction becomes large enough, a precipitate containing pectic acid and calcium ions. The maximal attraction is obtained

when there is one calcium ion for every two COOH groups, and if pectic acid is precipitated from a solution containing an excess of calcium such a "calcium pectate" is formed. The presence of calcium ions between chains aids greatly in orientation of the chains neatly parallel to one another, as is shown by the fact that dried calcium pectate is often double refracting.

In order to get a calcium ion to stay between two COOH groups, it is necessary to have a certain equilibrium concentration in the outside solution. If one increases the valence of the added cation this equilibrium becomes less (13) until in the case of a six valent cation it is negligible and practically every added cation is "adsorbed" on the pectic acid surfaces (7). We can make use of this fact to determine the charge density of pectic acid. If we add a very small amount of a salt having a six valent cation to a sol of pectic acid, a precipitate which is negatively charged as determined by cataphoresis will appear. As one adds more of the six valent cation the charge of the precipitate becomes zero and then positive. Now suppose that we take twice the amount of pectic acid. We will find that exactly twice the amount of six valent cation is needed to neutralize all of the charges of the pectic acid, and that, therefore, practically all of the added cation has been adsorbed. From the number of equivalents of six valent cation needed to bring about zero charge of a known amount of pectic acid we know at once the number of equivalents of charge on this amount of pectic acid. This method has been applied (7) and it has been shown that pectic acid is one of the most strongly negative colloids with which we are familiar, and that, furthermore, its negative charge is principally due to its carboxyl groups. It is because of this very high negative charge density that pectic acid is so readily precipitated by electrolytes, and this fact in turn has led several investigators to describe pectic acid as "insoluble" in water, their preparations presumably containing sufficient electrolyte to prevent dispersion.

Pectinic acids differ from pectic acid as we have seen in that only a portion of the carboxyl groups are free. Correspondingly, one finds that the negative charge density of pectinic acids is lower than that of pectic acid, and that pectinic acids are less readily precipitated by electrolytes. This charge density is, moreover, dependent to some extent on the acidity of the dispersion medium, in that it becomes slightly greater in solutions of smaller acidity. Greater

charge density in alkaline solutions results in easier precipitation by electrolytes in such solutions, a fact which has been observed several times (61, 7).

The carboxyl groups of pectic acid are thus of considerable interest as the principal source of colloidal charge. We should, therefore, examine them more closely. If one makes an electrometric titration of pectic acid, one obtains a curve which resembles superficially that of a monobasic acid. However, if one now calculates the dissociation constants along the curve, one finds that they increase constantly from about  $pK$  2.7 on the more acid side to over 4 on the less acid side (7). This variation of  $pK$  is continuous, that is, there are no "favored" ranges. We see at once that some characteristic of the pectin chain with which we are not as yet familiar, influences the strength of the carboxyl groups.

#### COLLOIDAL PROPERTIES

The pectins as a group have a large number of interesting colloidal properties. It is impossible to go into these properties here in detail, and only a few will be mentioned. Ordinary pectin is readily precipitated as a solid gel by the addition of a little dehydrating agent such as alcohol. This is a rather surprising fact, since related negative colloids such as gum arabic are not so precipitated. In fact, the entire classical theory of Freundlich and Kruyt (44) was based on charge and hydration as independent stability factors. However, there is good reason for this apparent deviation of pectin from the laws of the other bio-colloids. This reason is best made clear by an analogy. If one makes up a warm solution of 1 per cent agar and allows it to cool but prevents macroscopic gel formation by very vigorous agitation, one obtains a colloidal sol of agar particles, strongly negatively charged, and stable under ordinary conditions. This sol consists, however, of microscopic and submicroscopic gel particles and the addition of a small amount of dehydrating agent brings about gelification of the entire mass. Pectin also consists apparently of very small gel particles which, under normal conditions, are not quite able to coalesce to a uniform gel. This, however, is possible if the hydration of these particles is reduced even to a small extent (see also 22).

Pectins possess another interesting property which is possibly of considerable physiological significance. They are as we have seen

easily separated from the sol by the addition of dehydrating agents. In fact, they are so sensitive to dehydration that they are readily affected by other hydrophilic colloids, that is, they are "insoluble" in many of the other bio-colloids. A sol consisting of, for example, 8 per cent pectin and 8 per cent gum arabic, separates into two layers, one of which contains principally pectin, the other principally gum arabic. If a less strongly hydrophilic colloid, such as soluble starch is chosen, it is necessary to use a larger amount of it to bring about separation of a pectin layer. This phenomenon cannot be gone into in detail here, and it is sufficient to indicate that it may be of importance in the formation of pectin structures in the cell, for example, of the layered "slimes" of cinnamon, etc. (69).

To summarize the colloidal properties of the pectins: they are hydrophilic colloids of high negative charge, a charge which varies with the number of free carboxyl groups. This high negative charge is responsible for the ready precipitation of pectic substances by electrolytes, variations in the charge causing variations in this precipitability. The pectin sols differ from typical hydrophilic sols in that they are composed of larger particles, essentially gel fragments, and this is responsible for the ready precipitation of pectins by dehydrating agents. In fact, so sensitive is the pectin sol to dehydrating agents that dehydration may even be accomplished by other hydrophilic colloids.

#### DETECTION OF PECTINS

Methods for the localization of pectic substances are still relatively crude. Perhaps the best as yet discovered is the staining reagent ruthenium red (ammoniacal ruthenium oxy-chloride), introduced by that great student of the pectins, Mangin (46). This reagent stains pectins very deeply and does not stain other constituents of ordinary cell walls. It has, however, two disadvantages: *a*, it is not completely specific for pectins but rather for sixth carbon atom carboxyl groups; cellulose which has been subjected to a mild oxidation stains with ruthenium red, as do other of the "plant slimes" which contain such carboxyl groups; *b*, it does not differentiate between protopectin, pectin, and pectic acid or calcium pectate. This is a great disadvantage as will be shown below. Other methods for the microscopic localization of pectins were introduced by Mangin:

1. Removal of protopectin by hydrolysis with dilute acid, leaving the cellulose thus in place.
2. Removal of pectates, particularly calcium pectate by hydrolysis with dilute alkali.
3. Removal of cellulose with Schweizer's reagent, leaving pectins in the form of insoluble copper salts.
4. Solution of all pectic substances in warm ammonium oxalate.

By use of these methods in conjunction one can obtain a good idea of the localization of pectic substances in general, and even, more specifically, of calcium pectate. Discrimination between the different grades of pectinate is, however, impossible.

#### PECTIN IN FRUITS

Protopectin occurs most richly in the primary cell wall, that is, in the nomenclature of Kerr and Bailey (39), in the two thin layers first laid down upon the two sides of the middle lamella. The walls of most parenchymous cells consist of such pectin-rich primary walls, as do the walls of meristematic cells. The pectin which is obtained commercially from apples comes to a great extent from primary cell walls of the fleshy parenchyma. The "albedo" of oranges and of lemons also consists of parenchymous cells whose primary walls are extraordinarily rich in protopectin. Protopectin is not, however, confined to the primary cell wall. Its occurrence in the secondary walls of collenchyma, for example, is common. Anderson (1) has shown that the thickened walls of the collenchymous cells in the stems of tomato consist of alternate layers of protopectin and cellulose. The outermost layer of the root hair is also said to consist of a pectic substance, although its exact nature and function is not known (57).

Protopectin may remain unaltered in the cell wall in which it is laid down. Thus Anderson (2) has shown that protopectin is formed in the primary walls of wood (black locust), and that it remains even in the old wood. It may, on the other hand, undergo marked changes. Ehrlich (24) has suggested that protopectin is converted to lignin, but this would seem improbable from the very different natures of the two substances. More likely is the suggestion that protopectin may be changed under some conditions to hemicellulose. It has been shown above that conversion of protopectin to araban, a "hemicellulose", might be expected, and Ehrlich has in fact shown that araban is always present to some extent in tissues containing protopectin. Buston (15) has also presented

evidence to show that protopectin may be changed to hemicellulose as tissues become older, and Norman and Norris (53) have converted pectin to hemicellulose-like substances by mild oxidation *in vitro*.

The most striking alteration undergone by protopectin is, however, its degradation during the ripening of fruits. This was first worked out in detail for the apple by Carré (16, 17) and Carré and Horne (19), although it had been observed both by Fremy (27) and by Mangin (46). The protopectin present in the fruit rises during maturation until a final high level is reached which is maintained for some time. During storage of the fruit the protopectin then begins to decrease and decreases rapidly for several months, less rapidly for five or more further months. This decrease of protopectin is attended by a simultaneous increase of soluble pectin, and the inference seems fair that protopectin has been converted to soluble pectin. This supposition is further supported by the finding of Carré (18) that there is an enzyme present in the apple tissue which can convert protopectin to soluble pectin. Further changes in the pectin may occur, particularly in markedly overripe fruits. The pectin is in this case hydrolyzed still further, presumably to galacturonic acid, galactose, and arabinose, in any event to substances no longer recognizable as pectin. Similar changes have been observed in the pear (19) and in citrus fruits (31).

#### PECTINS IN THE MIDDLE LAMELLA

It was early recognized that pectic substances are present in the middle lamella. Thus Payen (56) regarded pectins as a sort of cement between the neighboring cells of a tissue. Mangin (46) realized particularly clearly that the middle lamella contains no cellulose but that it is rich in a pectic substance different from protopectin. This substance is not affected by boiling with dilute acid, the treatment used for removal of protopectin from the primary cell wall. It may be removed, however, by heating with dilute alkali or by precipitation of calcium present in the tissue with ammonium oxalate. Mangin, therefore, suggested that the pectin of the middle lamella is calcium pectate. That the middle lamella is actually rich in calcium was shown by Molisch (51), and that pure calcium pectate possesses solubilities *in vitro* similar to those of the middle lamella has been shown by Bonner (6, 7). The

calcium pectate nature of the middle lamella has, however, been contested by Tupper-Carey and Priestley (68), who maintained that it has the nature of a protein-pectin complex. That such complexes with protein may be formed by pectinic acids and by pectates has been shown by Bonner (7), but such complexes do not in any way resemble in their properties the middle lamella. Sloep (59) believes the middle lamella to be composed of protopectin, but, as shown above, the pectin of the middle lamella is greatly different from protopectin in its sensitivity to acid. It would seem at present that the calcium pectate theory of the middle lamella is supported by the most evidence.

The middle lamella also undergoes changes during aging of the cell wall. Carré (17) has shown that as apples become overripe there is a continuous decrease in the amount of pectic substance present in the middle lamella. At the same time the parenchymous cells become loosened or completely free from one another. The formation of intercellular spaces in general is in fact a phenomenon closely related to the pectate of the middle lamella. The surface of the cell wall abutting on the intercellular space retains a thin covering of its pectic material, and this may later be swollen into a projecting knob or wart. These have been studied in detail by Mangin (46) and by Vidal (72), as well as by Kisser (41). The knobs in the leaf spaces of *Dieffenbachia* have been carefully studied by Kisser and shown to consist of a thin tough membrane filled by a "swellable" substance. Both membrane and contents are of pectic nature, and experiments of the author have indicated that the difference between them is one of calcium content, the membrane being rich in calcium and probably approximating calcium pectate in composition, while the interior pectic substance is a pectinate very poor in calcium.

Among the commercially important processes which depend upon the dissolution of the middle lamella might be mentioned the retting of flax, which will be discussed later.

There can be no doubt as to the function of the pectates of the middle lamella, since they certainly act as a cement between the two adjacent primary walls. If this cement is removed the cells separate. It is interesting to note that calcium pectate can function also as a cement between two cellulose surfaces *in vitro*. As to the function of protopectin there is no such obvious explanation.

Since the pectins are relatively "hydrophilic" substances, it has sometimes been supposed that their presence is necessary for the hydration of young growing cell walls. Buston (15) has shown that pectins are most richly developed under conditions combining rapid growth and copious water supply. This does not of course show that the development of pectins is necessary for rapid growth, and there has been in fact no direct evidence to this effect. A widely held belief as to the function of protopectin is that it acts as a "cement" between ultra-microscopic blocks or "micelles" of cellulose. This view has recently been further developed by Farr and Eckerson (26), with the difference that they believe the cemented particles to be of microscopic dimensions. All such theories are based, however, upon the concept of the cell wall as composed of separate "bricks" of cellulose. It is now certain, however (70, 5, 30), that the cell wall is not built up in this way, but that the cellulose units or micelles are held together by cellulose molecules, and that protopectin functions only as an "intermicellar" substance, that is, that it forms its own system of long interlocking molecules in the meshes of the similar system of cellulose molecules. It seems not unlikely that the formation of poly-galacturonic acid derivatives by the cell is principally an indication of a certain level of oxidative activity in general met with only in young cells. This simple view is to some extent complicated by the above mentioned observation of Buston that the water balance of the cell also influences the formation of pectic substances. The oxidative level of the cell is again, however, related to the water content (8).

#### PECTIC ENZYMES

There are at least three types of enzymes which are responsible for the interconversion and degradation of pectic substances. These distinct types have been given different names by different investigators (Table 1), but at present the widely accepted nomenclature of Davison and Willaman (21) should be used, even though this nomenclature does not agree strictly with that of the pectic substances themselves.

The first pectic enzyme is *protopectinase*. Under this classification are included: *a*, protopectinases proper, which split protopectin of the cell wall to soluble pectin; and *b*, enzymes which attack the middle lamella with resultant maceration of tissue. The second



TABLE 1

|                             |   |  |   |
|-----------------------------|---|--|---|
| Present Name—Protopectinase |   |  |   |
| Names previously used       | protopectinase<br>Sloep   | pectosase<br>Carré (Branfoot)          | pectosinase<br>Bourquelot and<br>Herissey   |
| Present Name—Middle Lamella |   |  |   |
| Names previously used       | Protopectinase<br>pectinase<br>Sloep<br>Ehrlich<br>Davison and<br>Willaman                      | Paton<br>Jones<br>Harter and<br>Weimer | pectosinase<br>Beijerinck and<br>van Delden |
| Present Name—Pectinase      |   |  |   |
| Names previously used       | pectinase<br>Bourquelot and<br>Herissey<br>Sloep<br>Carré (Branfoot)<br>Davison and<br>Willaman | pectolase (?)<br>Ehrlich               |   |
| Present Name—Pectase        |   |  |   |
| No other name in use.       |   |  |   |

pectic enzyme is *pectinase*, which hydrolyzes soluble pectin, pectic acid, or pectates, to galactose, arabinose, and galacturonic acid. Lastly, there is *pectase*, which attacks only soluble pectin and pectinates, splitting off methyl alcohol, and forming pectic acid.

Protopectinase was first discovered by Bourquelot and Herissey (9) as an enzyme capable of converting protopectin to soluble pectin. Carré (18) has more recently shown definitely that such an enzyme is present in apple and in rutabaga tissue, and has shown that soluble pectin is the product of its activity. She also (12) stresses the difference between this true protopectinase and the enzymes of group *b* which destroy the middle lamella. It is not known, in general, whether the end product from the action of this type of enzyme is pectin, pectic acid, or degradation products as in the case of pectinase. Furthermore, it has often been found as in the case of Harter and Weimer (34) that enzymes of this class attack the middle lamella but leave the primary wall untouched. Until the middle lamella-destroying enzyme has been further studied it is, therefore, desirable to differentiate it sharply from true protopectinase.

Enzymes attacking the middle lamella are most commonly met with among the phyto-pathogenic fungi and bacteria and undoubtedly play a rôle in the maceration of plant tissue by these organisms

(37, 38, 35). In addition to the enzymatic attack of tissues there may be, however, an action of organic acids or of oxalates produced by the organism (60). Middle lamella protopectinase is found also in pollen, and Paton (55) suggests that its presence there is necessary for the penetration of the pollen tube between the cells of stylar tissue. The abscission of leaves is possibly due to a middle lamella-protopectinase mechanism (58, 59). The softening of fruits during the ripening process is undoubtedly in part a result of middle lamella-protopectinase action. Sloep has shown that in the case of fruits of *Mespilus*, ripening is accompanied by enzymatic dissolution of the middle lamella and consequent softening of the tissues.

Bourquelot and Herissey (10) also were the first to demonstrate the existence of pectinase. Their enzyme, from germinating barley, split calcium pectate to reducing substances, which we now know to have been galactose, arabinose and galacturonic acid, and later investigators have used the estimation of reducing sugars as a quantitative measure of pectinase activity. Qualitative or semi-quantitative determination can, however, be made by estimation of the time necessary to "liquefy" a gel of calcium pectate. Pectinase splits, however, not only calcium pectate but also pectin and pectinic acids, and it is, therefore, presumably present in overripe fruits, the pectin of which is hydrolyzed to galacturonic acid and sugars. This enzyme, also, is best known from fungi and bacteria, particularly *Rhizopus tritici* and *Sclerotinia cinerea*, studied by Davison and Willaman (21). These workers were able to effect partial separation of the middle lamella enzyme and pectinase by fractional precipitation with alcohol, and they also found that *Sclerotinia cinerea* which does not produce demonstrable middle lamella enzyme, produces, nevertheless, large amounts of pectinase. In general, however, ability of a micro-organism to attack the middle lamella is associated with the production of an enzyme acting upon calcium pectate, and supports the view that the middle lamella is of this nature (see 3). That pectinase capable of attacking calcium pectate *in vitro* should not *always* attack the middle lamella might easily be due, for example, to a longer chain length or to impurities of the native material.

Pectase, the best known of the pectic enzymes, hydrolyzes the methyl alcohol from soluble pectin with the production of pectic acid, as shown first by Von Fellenberg (74). In the presence of

traces of calcium this pectic acid sets to a gel, and for this reason pectase is commonly known as an enzyme causing gelling of pectin, even though Bertrand and Mallevre (4), Goyaud (32), Kopaczewski (43), and Mehltz (47) have emphasized the rôle of calcium in the process. The action of pectase is essentially similar to that of the lipases, and Kertesz (40) has in fact shown that its action may be duplicated by lipase from *Ricinus* or even from the pancreas. To its lipase nature is probably due the fact that pectase is almost universally distributed in plant tissues, although no function of pectase as such has as yet been found.

#### THE RÔLE OF PECTIC ENZYMES IN THE RETTING PROCESS

Pectic enzymes are of practical importance in the retting process. The retting of textiles, particularly flax, has long been known (42) to be due to a solution of intercellular pectic substances and consequent separation of fibers from the ground tissue of the plant. Winogradsky (74) moreover demonstrated that retting is due to definite micro-organisms, one of which he named *Granulobacter pectinovorum*, which "ferment" pectic substances. Beijerinck and Van Delden (3) even produced artificial retting with *Bacillus subtilis*. More modern investigations (20) have shown that high quality of the retted product is dependent upon a relatively high pectin content, and that it is essential that not only the protopectin of the fiber walls be left, but that also the middle lamellae joining the individual fibers into fiber bundles must remain intact. Eyre and Nodder (25), who have investigated the retting process in great detail, divided it into four periods. During the first period soluble sugars are fermented by the mixture of micro-organisms present, and considerable carbon dioxide is evolved. During the second stage some pectin is attacked by pectinases with the production of organic acids in the retting solution, but no solution of the middle lamellae is apparent. In the third stage a rapid decomposition of the middle lamellae sets in, particularly of the cortical cells surrounding the fiber bundles. Considerable quantities of organic acids, presumably galacturonic acid, appear in the retting solution. During the fourth stage the pectic substances of the fiber bundles themselves are attacked, with consequent decreases in strength and quality of the fiber. It is, then, necessary to stop the ret after the completion of the third stage, or after about 190 hours under the

conditions of Eyre and Nodder. Several pectic enzymes must apparently take part in the retting process. During the second stage pectinase appears to be the chief agent, attacking soluble pectins of the tissue. During the third stage a middle lamella enzyme as well as pectinase appear to be active, and during the fourth stage a true protopectinase attacking protopectin of the fibers is probably added to these. It is possible that the acids formed in the retting solution by the metabolism of the micro-organisms also contribute to the degradation.

#### PECTINS IN JAMS AND JELLIES

By far the most important use of the pectins, aside from plant functions, is their part in the making of jams and jellies. There is a very voluminous literature on this practical subject and there is in addition no satisfactory, unified theory of the jelly formation. A short summary of the principal factors must, therefore, suffice.

It is necessary to emphasize the fact that the calcium pectate gel and the pectin sugar jelly are fundamentally different. The former is obtained when calcium ions are added to a sol of pectic acid or of a soluble pectate, and cannot be made with pectin itself. The latter is never obtained with pectate but only with pectin and certain pectinates. After the discovery of the pectin sugar jelly by Brannon (11), its properties were studied by Fremy (28, 29) (who misinterpreted the jelly as pectic acid), Von Fellenberg (74), and Sucharipa (64) (among many others), and it was generally recognized that there are three principal factors involved in jelly formation: pectin, sugar, and acid. For the formation of a good jelly, pectin and sugar in suitable concentrations must be brought together at the appropriate acidity. The temperature at which this is effected is of some importance but as a matter of convenience, the boiling point of the solution is often used. Excessive heating, however, reduces the strength of the jelly.

Protopectin is incapable of forming sugar jellies, and for this reason green fruits containing much protopectin and little pectin are not suitable for jelly-making unless the protopectin is first hydrolyzed by boiling with dilute acid. Since pectic acid does not form a sugar jelly, it is also necessary to avoid alkaline solutions as well as the action of pectase from the fruit juice. As to the amount of pectin necessary, this varies greatly with different preparations

and modes of treatment of the protopectin. With a good pectin it can be as little as two tenths of one per cent in the final jelly, with a poor pectin as much as one and one half per cent. The properties of pectin which affect its jellying power were first studied extensively by Von Fellenberg (74). He suggested that high jellying power depends upon high methyl alcohol content, and this view has been widely accepted. Lüers and Lockmüller (45) for example found that pectins with a methoxy content of less than seven and three tenths per cent do not jell at all while those with a methoxy content of ten to twelve per cent jell very well. Meyers and Baker (49, 50) have, however, reinvestigated this question and have shown that the parallelism is probably secondary and merely accompanies other changes in the pectin which are of more direct importance. Decreasing methoxy content is accompanied by decreasing viscosity of the pectin sol (74) and viscosity is, as shown by Meyers and Baker, definitely related to jellying power. With decrease of viscosity of a given pectin (by boiling for different times) a parallel decrease of jellying power is brought about, and this relation holds from low viscosities to relative viscosities of about fifteen. Above this point increase of viscosity is not attended by any increase in jellying power. Among different samples of pectin the relation does not hold so well, due apparently to variations in galactose-arabinose content of the pectin. Removal of galactose and arabinose increases jellying power but decreases viscosity. From this work of Meyers and Baker we can, however, definitely say that jellying power does depend upon "degree of polymerization" of the pectin, that is, upon the length of the pectin chains.

The amount of sugar used in preparation of the jelly may vary within wide limits, depending on the grade of pectin, the pectin concentration, and the hydrogen-ion concentration. The concentration in the final jelly is much more nearly constant and is approximately saturated (sixty-five to seventy per cent), even though with very pure pectin. Tarr and Baker (67) have been able to prepare jellies without the addition of sugar. The ratio of pectin to sugar is of great importance in the determination of the strength and texture of the jelly. High sugar-pectin ratios lead to a soft jelly; low ratios to a tough jelly. The particular ratio needed depends, moreover, on the "degree of polymerization" of the pectin used, that is, the longer the pectin chains the more sugar in relation to pectin is

necessary for the production of a soft jelly. If pectin of constant chain length is used, then the higher the concentration of pectin the firmer the jelly. The ratio of pectin to sugar necessary for the production of a jelly of a given strength also decreases as the hydrogen-ion concentration decreases from pH 3.4 to 3.1 (67).

The sugar factor in the pectin-sugar jelly is then rather complex and depends upon many variables. Although it is commonly believed that sugar acts only as a dehydrating agent for the pectin (see 54), still the existence and significance of these variables are not as yet adequately explained.

The presence of acid has long been known to be essential for jelly formation, but Tarr (66) first showed that it is not the quantity of acid but the acidity (pH) which is the controlling factor. The optimal pH of jelly formation is in the region 3.2 to 3. Acidities below this give weak jellies, above this, "syneresis" or expressing of liquid. With increasing pectin concentration the optimal pH shifts slightly downward, which it does also with increasing sugar concentration. Some pectins possess sufficient free acid groups to bring the jelling mixture to the correct pH, and in such cases the addition of more acid is unnecessary. Natural fruit juices also often contain sufficient acid. With fruit juices which possess sufficient pectin and sugar but insufficient acid, such as certain peaches and pears, jelly formation can be brought about by the introduction of acid. The function of acid is also not well understood although it has been proposed that it has principally to do with reduction of the pectin's negative charge, thus making approximation of the pectin units easier (59). If this should be the case, one would expect that other highly charged cations should have a similar effect. Critical experiments have not as yet been made, but the investigations of Von Fellenberg and of Halliday and Bailey (33) indicate that calcium may at least partially take the place of hydrogen-ion. It is well known that the jelling power of natural fruit juices varies greatly. Juice from overripe fruit fails to jell since the pectin has been destroyed by the action of pectinase. Green fruit or fruit rich in protopectin but poor in pectin demands long cooking in slightly acid solution to bring about hydrolysis of protopectin to pectin. In some cases (green apples, lemons, some grapes) the fruit itself contains sufficient acid to bring about this hydrolysis, in others acid must

be added (peaches, pears, figs, melons). Rhubarb on the other hand contains sufficient acid but insufficient pectin of any kind, and strawberries are deficient in both acid and pectin.

In conclusion it might be remarked that detailed instructions for the preparation of jellies from several different fruits may be found in a special report of the Delaware Agriculture Experiment Station.

#### CONCLUSION

It has been attempted to show that the pectic substances as a group are of interest from the most varied points of view, and that all these points of view have contributed to our present knowledge. The structure and the colloidal behavior of the pectic substances are of interest to the chemist. In industry pectins are met with, either as filtering problems or as essential components of industrial products. But the pectins are, after all, plant products of the greatest interest to the botanist and to the physiologist who study their manifold functions in the plant, and the relation of chemical structure to these functions. From what has been said in the foregoing pages it is apparent, however, that for a complete understanding of pectic substances, much remains to be done, by both chemist and botanist.

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# THE PRESENT STATUS OF SEED TREATMENT, WITH SPECIAL REFERENCE TO CEREALS

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Man's unceasing struggle against the destructive forces of Nature is well illustrated by his war on plant diseases. Losses due to fungous and bacterial diseases of commercial crops in the United States amount to about a billion dollars annually (107). The annual loss caused by one disease, bunt of wheat, may amount to over 25 million dollars (38). During the 11-year period 1917-1927 smuts in oats caused an average annual loss of 50,000,000 bushels (119). These losses combined with those caused by other diseases of these and other crops, make a staggering total.

Much of this loss is preventable. The principal preventive measures include sanitation, crop rotation, and certain other cultural practices, the use of resistant varieties, and seed treatment.

The purpose of this paper is to sketch briefly the development of seed treatment, especially for cereals, and to describe the present problems, trends, and practices in that field as they apply to conditions in the United States.

No attempt will be made to review more than a small part of the prodigious amount written on the subject, or even to cite more than a small percentage of the articles perused. Some of the statements made are based on the writer's own experiments, data from which have not been published.

## HISTORICAL

Seed treatment for the prevention of plant diseases, especially those of cereals, has been practiced, in one form or another, for about three centuries.

At first, in the absence of definite knowledge concerning the nature of plant diseases, preventive measures were of a more or less superstitious nature, such as sowing in the dark of the moon, or sticking branches of laurel in the grainfields, "to draw the blighting vapors to them" (136). Some of the earlier materials used for treating cereal seeds were lime, salt, saltpeter, and wood ashes.

Copper sulfate probably was the first standard fungicide used, and its intelligent application dates back to 1761 (113). It did not come into general use, however, until a century later when Kühn's (65) experiments established a basis for making definite recommendations regarding its use. Later investigators made other recommendations concerning the use of copper sulfate, the most important of which was that, after treatment, the grain be dipped in lime-water to prevent seed injury. Despite the advances made in seed treatment methods in recent years, many farmers still use the copper sulfate treatment for the control of bunt in wheat.

Another seed treatment method of early origin still in use is the hot-water treatment developed by Jensen (51) in 1887. It still is the only known treatment that will kill certain deep-seated fungi like that causing the loose smut of wheat (*Ustilago tritici*) which are not controlled by surface disinfectants. It also is used for treating certain vegetable seeds.

Formaldehyde was first advocated as a seed treatment in Germany by Geuther (32) in 1895 and in the United States by Bolley (6) in 1897. It still ranks among the foremost liquid treatments because of its cheapness and its general effectiveness, in spite of its tendency to injure the seed.

Copper sulfate and formaldehyde continued as the outstanding seed treatment materials up to about 1914. Mercuric chloride and other materials were tried but not generally recommended. In 1912 organic mercury compounds were introduced as seed disinfectants in Germany and in early experiments Riehm (104, 105) along with others found them effective in cereal-disease control. Among the first of these to be marketed was a chlorophenol mercury compound known as "Uspulun", placed on the market in Germany about 1915. Similar compounds under the trade names "Chlorophol" and "Semesan" soon appeared in the United States. These materials were used in solutions ranging in concentration usually from .25 to .75 percent. In general they were found very effective in cereal-disease control with little or no seed injury (68, 71, 106, 122, 126).

Dust disinfectants first came into prominence as a result of the work of Darnell-Smith with copper carbonate (18, 19) in Australia in 1915. Due to certain apparent advantages this form of seed treatment met with immediate popularity and started the era

of dust fungicides. At first the use of dust fungicides was restricted to the control of diseases due to surface-borne organisms such as bunt of wheat; but experiments soon showed that the more deep-seated organisms, like those causing the smuts of oats (27) and covered smut and stripe of barley (73), could be reached by certain dust fungicides (69, 71, 72). From then on, liquid fungicides lost favor and dust fungicides gained in popularity, not only for treating cereal seeds but seeds of other crops. Although liquid fungicides have by no means been entirely discarded, yet if dust fungicides of equal effectiveness can be economically used, they usually are preferred.

#### ADVANTAGES OF DUST FUNGICIDES

Dust fungicides possess certain outstanding advantages over liquid fungicides.

1. They are more easily applied; the wet disagreeable task incidental to immersion treatments is eliminated as also is the work of drying the seed, and the danger of the seed freezing, sprouting, heating or moulding before it dries.

2. The use of dust fungicides greatly reduces the chances of errors in seed treatment. Liquid fungicides frequently are used in excessive concentrations or the immersion period may be too long and thus impair germination. On the other hand the solution may be too dilute or the period of immersion too short to effect disease control. Certain fungicidal solutions when used repeatedly have so much of their essential ingredients taken up by the seed that additional concentrated solution must be added occasionally (28, 64). Mistakes are easily possible in adding the chemical to replenish the solution.

It also is possible to apply too much or too little of a dust fungicide, but such errors are revealed to some extent by the appearance of the treated seed. The excess dust usually is readily discernible, while if an insufficient amount is applied its failure to coat the seed properly will be evident especially if the recommended rate per bushel is 2 ounces or more. Dusts that give off fumes and thus disinfect the seed largely before it is planted, must be applied with greater precision as to amount than dusts that are relatively inert until the soil moisture acts upon them.

3. Dust fungicides furnish greater protection against recontamination of the seed.

4. They protect the seed, to some extent, against soil organisms.

5. They protect the seed against weevils and rodents (79).

6. Their application is largely independent of temperature while low temperatures decrease the effectiveness of some liquid fungicides (31, 66, 79).

Dust fungicides are not without certain disadvantages. The fine dust when inhaled may cause extreme discomfort or even illness. The vesicant action of mercurials is especially disagreeable. With the exception of formaldehyde, liquid fungicides usually do not affect the operators while being applied.

Dust fungicides may interfere with the ready flow of grain through the drill, especially those dusts applied at the rate of 2 or more ounces per bushel of seed. Connors (13), for example, found that treatment with copper carbonate increased the bulk of wheat seed 7.7 percent and reduced its rate of flow about 12 percent. Insufficient or excess soil moisture (131) or the presence of certain organic materials in the soil (115, 116) may decrease the effectiveness of some dusts that do not take effect until after sowing.

Dust fungicides, as a rule, are more expensive than liquid disinfectants.

#### PROBLEMS IN DEVELOPING AND TESTING SEED TREATMENTS

The work of testing the relative effectiveness and practicability of different fungicidal materials presents certain problems to the investigator. The first of these relates to obtaining a supply of seeds carrying sufficient infective material to provide an adequate test for the fungicide. If the organisms are entirely surface-borne, as in bunt and flag smut of wheat, and kernel smut of sorghum, it is a simple matter to infest the seed. But in many cases the organisms are placed by Nature deeper within the seed (27, 73), in a manner that cannot be easily and surely duplicated artificially. The disparity in results obtained by different investigators may sometimes be explained by the fact that in one case naturally inoculated seed was used and in the other the seed was artificially inoculated. This makes it desirable to secure seed from a badly infected crop, or still better, if possible, seed from

the same lot from which the infected crop was grown. In some years, strange as it may seem, badly infected fields are not readily found. In other years, even though the infection may be high in the field, the weather or other conditions may not be conducive to a heavy invasion of the seed by the organisms (70). Such seed when used in seed treatment experiments yields disappointing results because it fails to test thoroughly the merits of the fungicides under study. Again, suitable seed may be available, but the conditions under which it germinates or the plants are grown may not favor development of the disease; thus another experiment may end inconclusively.

Effectiveness in disease control, however, is only one of the factors by which the relative practicability of a seed disinfectant is judged. We also must consider its "chemotherapeutic index" or margin of safety (31); its effect upon germination, stand, vigor, and yield; its corrosive effects on treating and sowing machinery (25); its injurious effects on the persons applying it or handling the treated seed; its stability, cost, and other features. For example, fungicidal dusts containing a considerable percentage of such materials as mercuric chloride or mercuric iodide are undesirable because of their corrosiveness and extremely poisonous nature. Many effective dusts have proved commercially impracticable because their high mercury content made the price prohibitive. The chief objection to certain volatile dusts is their rapid deterioration unless they are kept tightly sealed. Many effective materials, such as copper chloride, are too hygroscopic to retain the fluffiness desirable in a dust fungicide; others, like cuprous oxide, are not always chemically stable but gradually change to other forms unless specially treated.

A comparison between the large number of materials that have been reported effective in plant-disease control from time to time by various investigators and the relatively few commercially successful fungicides now available for effectively controlling these same diseases, seems to indicate that most of the substances found effective in an experimental way were found unsuitable in the manufacture of practicable fungicides, either because of undesirable physical or chemical properties or excessive cost. The production of experimental seed disinfectants was, for a time, a popular "side line" of many commercial concerns. The realization of the

fact that, like Rome, good practicable seed disinfectants cannot be built in a day, has resulted in fewer of these products being put on the market in recent years.

#### CEREAL DISEASES COMBATTED BY SEED TREATMENT

The different diseases that attack cereals will not be described here and only those will be mentioned that are wholly or in part amenable to control by seed treatment. Adequate descriptions of these different diseases will be found in a number of publications (8, 34, 40, 60, 61, 62, 77, 85, 120).

Some of these diseases, such as barley stripe, are associated with a single host; others, such as scab, are common to several cereal hosts.

General recommendations for seed treatments of wheat, oats, barley and grain sorghums in the United States are very briefly outlined in a recent Federal publication (52). More detailed information on diseases or materials and methods for their control is available in other publications (4, 5, 7, 8, 16, 34, 35, 41, 61, 62, 77, 85, 89, 127, 134).

Wheat probably has received more study from the standpoint of seed-borne diseases and their control than has any other cereal. Orton (92) lists over 50 organisms that have been isolated from wheat seed by various investigators. Henry (39) isolated species of fungi representing between 15 and 20 genera. Among these the most common were species of *Alternaria*, *Fusarium*, and *Helminthosporium*. In recent studies on light-weight wheat the writer isolated species of *Alternaria*, *Helminthosporium*, *Fusarium*, *Penicillium*, *Aspergillus*, *Rhizopus*, *Macrosporium*, *Cladosporium*, and bacteria from the seeds. Most of these have been isolated also from other cereal seeds. The presence of these organisms on seeds does not necessarily indicate that they are pathogenic. Most of them, however, are at least capable of attacking the food material in the germinating seed and thus preventing or inhibiting germination. To some extent this can be prevented by seed treatment, a fact that applies to the other cereal seeds as much as it does to wheat.

The principal seed-borne diseases of wheat, known to be wholly or partly amenable to control by seed treatment, are: bunt (*Tilletia tritici* and *T. levis*), flag smut (*Urocystis tritici*), loose smut



(*Ustilago tritici*), seedling blights, caused by certain species of *Fusarium* and *Helminthosporium*, black chaff (*Bacterium translucens* var. *undulosum*), basal glume rot (*Bacterium atrofaciens*) and anthracnose (*Colletotrichum graminicolum*).

Loose smut can be controlled only by the hot-water treatment, which, if applied, also eliminates the other seed-borne diseases. The latter, however, insofar as they are caused by seed-borne organisms, generally respond to treatment by the better organic mercury compounds, such as ethyl mercuric phosphate. Formaldehyde, although not used so widely as formerly, also is effective, but, at times, its benefits do not compensate for the injury it causes. The better copper dusts, such as copper carbonate, basic copper sulfate, and copper oxychloride will prevent bunt and flag smut (20) in the absence of soil infestation, and will partially control some of the other diseases mentioned. They also afford some protection against soil-borne organisms, but are less effective in this respect than the better organic mercurials.

Rye is somewhat susceptible to some of the diseases mentioned under wheat. Among these are bunt, loose smut, anthracnose, and the seedling blights caused by species of *Fusarium* and *Helminthosporium*. Rye also is attacked by stem smut (*Urocystis occulta*), and by bacterial blight caused by *Bacterium translucens* var. *secalis*. None of these diseases, except possibly stem smut, is serious in the United States, and seed treatment of rye is not generally practiced. In Europe the disease known as snow mold "Schneesimmel" (*Fusarium nivale*) is very important and generally is combatted with organic mercurials. The treatments recommended for wheat also may be used for rye.

The important seed-borne diseases of barley are: covered smut (*Ustilago hordei*), brown loose smut (*U. nuda*), black loose smut<sup>1</sup> (*U. nigra*), stripe (*Helminthosporium gramineum*), anthracnose (*Colletotrichum graminicolum*), bacterial blight (*Bacterium translucens*), and the seedling blight stages of net blotch (*Helminthosporium teres*), spot blotch (*H. sativum*), and scab (*Fusarium graminearum*). As in the case of wheat, the hot-water treatment is the only one that prevents brown loose smut (*U. nuda*). The better organic mercurials, such as ethyl mercuric phosphate, are most effective against the others, especially stripe. Formaldehyde

<sup>1</sup> Also called by some false loose smut or intermediate loose smut.

in liquid or dust form will control the covered and black loose smuts fairly well and also will reduce considerably the amount of seedling infection caused by other seed-borne organisms. Copper dusts, on the whole, are relatively ineffective against barley diseases except in those varieties having hull-less seeds.

The seed-borne diseases that affect oats are: covered smut (*Ustilago avenae*), loose smut (*U. levis*), leaf spot (*Helminthosporium avenae*), anthracnose (*Colletotrichum graminicolum*), halo blight (*Bacterium coronofaciens*), bacterial stripe blight (*B. striafaciens*) and the Fusarium seedling blights caused by such organisms as *F. graminearum*, *F. culmorum*, *F. avenaceum*, and *F. nivale*. Organic mercurials, such as ethyl mercuric phosphate and others (90, 137), formaldehyde liquid, and formaldehyde dust disinfectants, are widely used for disinfecting oat seed. They are named above in the order of preference. As in the case of barley, copper dusts are effective only on the seed of hull-less varieties.

The name sorghum, used in its broadest sense, includes groups known as broomcorn, darso, durra, feterita, hegari, kafir, kaoliang, milo, sorgo, sweet or saccharine sorghum, and Sudan grass. There also are numerous crosses of the above groups. The most common seed-borne diseases of sorghum are covered kernel smut (*Sphacelotheca sorghi*) and loose kernel smut (*S. cruenta*). Head smut (*Sorosporium reilianum*) usually is brought about by soil infestation, but it is possible that the seed may carry the disease into new areas (92). Anthracnose (*Colletotrichum fulcatum*) also may be carried on the seed, although, like head smut, this is not the chief way in which it is spread. Certain bacterial diseases also are suspected of being seed-borne, chief of these being bacterial streak (*Bacterium holcicola*), bacterial stripe (*B. andropogoni*), and bacterial spot (*B. holci*). Seedling blights and seed rots frequently are caused by species of *Penicillium*, *Fusarium*, *Aspergillus*, *Rhizopus* and other genera borne on the seed or present in the soil.

In inoculation experiments with a number of organisms of the above genera, *Penicillium oxalicum* was found capable of causing an especially severe seedling blight of sorghum, and *Fusarium culmorum* attacked the seed and prevented germination.

The smuts and other diseases of sorghum insofar as they are seed-borne can be prevented by the organic mercurials previously mentioned, copper carbonate, sulfur, and other common surface disinfectants in dust form. Formaldehyde and copper sulfate solutions are no longer recommended because of the danger of seed injury. (21, 22, 55). Losses from seed rots and seedling blights, caused largely by soil organisms, can be greatly reduced by copper and organic mercury dusts. The damage to seed caused by these organisms varies with soil conditions and with the thickness of the seed coat (117).

Proso and foxtail millets also are attacked by sorghum kernel smuts in addition to smuts peculiar to these two grasses, (*Ustilago panici-miliacei* and *U. crameri*, respectively). The treatments recommended for sorghum are suitable also for the millets.

Rice is a cereal in the culture of which seed treatment plays a very minor part. Most of the important rice diseases are soil-borne, and seed treatment has little or no effect on their incidence. The hot-water treatment has been recommended for seedling blight caused by species of *Helminthosporium* (77, 125) and a modified mercuric chloride treatment for seed-borne diseases of rice in general (75). Tullis (letter) states that seed-treatment with cuprous oxide and formaldehyde reduces the number of seedlings infected with *Curvularia lunata* and that 2 percent ethyl mercuric chloride reduced infection by *Helminthosporium oryzae*. However, he stated that no seed treatment was wholly effective against soil-borne organisms, and none caused significant increases in yield, and, therefore, seed treatment of rice is not recommended as a practical farm procedure in the United States.

Flax is subject to a number of diseases that are caused by soil-borne organisms and, therefore, not controlled by seed treatment. Chief among these is wilt (*Fusarium lini*). The pasmo disease (*Phlyctaena linicola*), anthracnose (*Colletotrichum linisolum*) and browning or stem-break (*Polyspora lini*) may be seed-borne, although all are largely spread by other agencies. Various seedling blight and damping-off organisms also may be seed-borne.

Seed treatment of flax has not been widely practiced. The recent literature on the subject has been reviewed by Flor (23). The latter in 4 years' experiments found seed treatment of flax with copper carbonate, organic mercurials, and formaldehyde did

not benefit consistently either stand or yield. These results are not in agreement with others obtained in Iowa (10, 101) where considerable increases in yield followed the use of disinfectants containing ethyl mercuric chloride and phosphate. Flor (23) attributes this disparity in results to differences in climatic factors and soil flora in North Dakota and Iowa.

The chief seed or seedling diseases of corn combatted by seed treatment are those caused by *Diplodia zeae*, *Gibberella saubinetii*, *Basisporium gallarum*, *Fusarium moniliforme*, *Penicillium oxalicum*, *Aspergillus niger*, *A. flavus*, and other species of *Penicillium*, *Rhizopus*, and *Fusarium* (43, 63). Orton (92) names organisms representing 20 genera that may be seed-borne but some of which cannot be controlled by seed treatment. Only seedling diseases of corn are successfully combatted by seed treatment (61). Other diseases such as ear rots, smut, stalk rots, leaf blight, root rots, and bacterial wilt are not eliminated by seed treatment.

Different investigators are not entirely agreed regarding the advisability of treating seed corn every year. This is probably on account of differences in soil and climatic conditions in different parts of the country. In certain experiments in Kansas (82), Arkansas (81), and Nebraska (58, 59), no consistent benefits followed the use of seed disinfectants for corn, while in certain other States farther east and north seed treatment proved beneficial and is recommended as a good farm practice (43, 63, 84, 98, 100, 102, 103).

In extensive seed-treatment studies made in connection with the Iowa corn yield test in 1933, 1934, and 1935 (108, 109, 110), seed treatments frequently improved stands, but significant yield increases were obtained only from hybrid seed, and then only in certain sections and in certain years. In no case did seed treatment of open-pollinated seed cause significant increases in yield. This seed, it is thought, may have been more carefully selected and hence of better quality than the hybrid seed.

In general it has been found that good corn seed that is found relatively free from disease-producing organisms when tested in the seed germinator, is not benefited by seed treatment unless sown in cold wet soil. In the latter case, good fungicidal dusts protect the seed to some extent from soil inhabiting organisms.

Seed, infected to any considerable degree with the organisms mentioned above, especially *Diplodia zeae*, usually is benefited by seed treatment and particularly if cold wet weather follows planting. Much of the injury due to soil-borne organisms can be reduced by proper cultural practices (63). Corn seed treatment is not a substitute for careful seed selection and seed testing, but may be regarded in the same light as crop insurance. Whether or not benefits will accrue from corn seed treatment frequently depends upon factors or conditions that vary from year to year and are beyond the control of the grower.

The liquid fungicides formerly recommended for treating seed corn have been discarded in favor of fungicides in dust form. Some of the earlier dust treatments, such as Sterocide, Bayer Dust and others, have already become obsolete. The current treatments most frequently suggested in recent investigations are New Improved Semesan Jr. (successor to Semesan Jr. and Improved Semesan Jr. but changed in composition), Barbak 111, and Merko. All of these contain mercury. Copper fungicides on the whole have been found unsatisfactory (82) as corn seed treatments.

#### FUNGICIDAL MATERIALS

The foregoing discussion of the principal diseases of cereals amenable to control by seed treatment may well be followed by a brief appraisal of the fungicides generally used for their control. These materials may be arranged into several general groups:

1. Non-metallic substances such as formaldehyde and related materials, including formaldehyde dusts, paraformaldehyde dusts, and mixtures of formaldehyde with other materials, sulfur, hot water, hot water and alcohol, calcium and sodium compounds, phenols, etc.
2. Copper compounds, mostly inorganic, such as copper carbonate, sulfate, oxide, chloride, oxychloride, acetate, oxalate, phosphate, arsenate, and others.
3. Mercury-containing compounds, mostly organic, but also some inorganic.
4. Salts of other metals, such as zinc, nickel, lead, etc.

Formaldehyde (liquid) used as a soak, dip, sprinkle, or spray still retains considerable popularity as a fungicide. In cereals it is used mostly for the control of the smuts of oats, and bunt of

spring wheat, although it also is used to some extent for treating barley, sorghum, and other seeds. For bunt control it is best used as an immersion treatment, so that the bunt balls, light kernels, and trash can be skimmed off. After immersion in a 1:320 solution the wet seed is drained, covered for several hours, and then spread out to dry sufficiently to be run through the drill. If the seed is cleaned so that it contains no bunt balls, other methods of application may be used. The spray method is the most popular for oats (37). One pint of formaldehyde diluted with 1 of water is sprayed on 50 bushels of oats as the latter are shoveled over. The sprayed oats are covered from 5 to 8 hours and then sown, no drying being necessary. In this, as in all other treatments with formaldehyde, the seed should be sown as soon as possible after treatment to prevent injury.

The chief advantages of formaldehyde are its cheapness and its general effectiveness if properly applied. Unlike many other fungicides, it does not render the seed unfit for feed and this is a decided advantage. Its great disadvantage is its narrow margin of safety and the many factors that make it injurious to the seed (49). It not only fails to protect the seed against soil organisms but makes it more susceptible to them (76). Frequently, although it eliminates a considerable percentage of disease from a crop, it reduces the yield below that obtained from untreated seed.

Formaldehyde dust was designed to make available for seed treatment the fungicidal effectiveness of formaldehyde without its attendant disadvantages (111, 112). It consists of an inert material, like talc, charcoal, kaolin or infusorial earth, which has been allowed to absorb 4 to 8 per cent by weight of commercial formaldehyde. The finished product must be kept tightly sealed to have it remain effective. It is used chiefly to combat the smuts of oats (*Ustilago avenae* and *U. levis*) and, to some extent, covered smut (*U. hordei*) and black loose smut (*U. nigra*) of barley. However, since it does not control barley stripe (73) it is not generally recommended for that cereal. It is applied to grain at the rate of about 3 ounces per bushel, after which the grain is sacked and allowed to stand for several hours or overnight. Prolonged storage after treatment may impair somewhat the viability of the seed. The extent of this injury varies with different varieties of grain and also with the moisture content of the seed,

the relative atmospheric humidity, and the conditions under which the seed is stored.

Oats or barley may be treated with formaldehyde dust for about 3 to 5 cents per bushel. This dust is easily applied, it usually causes no serious injury if the treated seed is stored properly and not too long, it does not seriously affect persons applying it and it does not render the treated seed wholly unfit for feed.

Its greatest disadvantage is its rapid deterioration when it is exposed to the air and the resulting disappointing results frequently obtained with it. As a rule, it does not significantly increase yields from clean seed and occasionally depresses yield (62, 74, 137). Since the formaldehyde spray effectively controls oat smut, is easily applied, and is much more dependable and cheaper, there is little argument for the use of formaldehyde dust for this purpose. It fails to control stripe in barley and, therefore, cannot be considered an ideal material for treating that cereal. It is not effective in bunt control and is not recommended for sorghum. It satisfactorily prevents damping-off and other seedling diseases of certain vegetables and ornamentals (1), and is now widely used in combatting them (133).

Paraformaldehyde occasionally has been tried as a fungicide, but with little success. Recently, the writer obtained promising results in oat-smut control with Formacide, a recently developed paraformaldehyde dust. According to the manufacturers, this dust contains a catalytic agent that, in the presence of moisture, causes the paraformaldehyde to revert to the gaseous formaldehyde. One of its apparent disadvantages is that treated seed cannot be stored a long time without injury. Further experiments with this material are necessary to determine its possibilities and limitations. Mixtures of liquid formaldehyde with mercuric chloride and other materials, as a rule, have not proved satisfactory for seed treatment.

Sulfur has been recommended as a promising fungicide for the control of covered kernel smuts of sorghum and closely related crops (55, 83, 129). Its use also has been suggested for the control of barley covered smut (56, 87). In experiments by the writer it was ineffective in controlling smuts of wheat (68), oats, or barley. It is possible that a relatively high temperature may increase the fungicidal effectiveness of sulfur by causing it to

volatilize more rapidly (93). This would make it more effective as a seed disinfectant if applied during very hot weather, although Horsfall's (45) experiments indicate otherwise. Its fungicidal properties are demonstrated by its control of cereal rusts (78), powdery mildew, potato bacterial wilt and other diseases. It is cheap and relatively harmless to seed, machines, and operators. If there could be found an oxidizing or other agent that would increase its effectiveness in seed treatment (67, 121), it would be a valuable addition to the present supply of cereal seed disinfectants.

The hot-water method of treating seed (29, 30, 36, 42, 91, 95, 118, 130) is not widely advocated because of the tediousness of applying it and its narrow margin of safety. However, it remains the only practicable means of controlling the loose smuts of wheat (*Ustilago tritici*) and barley (*U. nuda*), and is employed occasionally when smut-free seed of these cereals is not available. Usually, only enough seed is treated to sow a seed plot that will supply smut-free seed the following year. Attempts have been made with varied success to lower the temperature and lessen the time of immersion necessary for loose smut control by adding about 3 percent alcohol to the water (29, 30, 91). Frequently, the hot-water treatment seems to lower the percentage of germination, especially if the seed is tested shortly after treatment. Germination, however, may be better several weeks after the seed has been treated. Alpha barley, for example, germinated 63 percent 2 days after the hot-water treatment had been applied and 83 percent two weeks later. Untreated seed of the same lot germinated 90 percent. It has been found that copper carbonate sometimes improves the germination of wheat previously subjected to the hot-water treatment (36).

Copper and mercury appear to be the chief metals employed in seed disinfectants. Certain salts of nickel, lead, and zinc have been found effective at times in the control of some cereal diseases but have never been widely utilized for that purpose. At present zinc oxide is being advocated to combat post-emergence damping-off (46) in vegetables and ornamentals. For this purpose it is applied to the soil rather than to the seed. It also has been used successfully, however, as a seed disinfectant for spinach and tomato seed (14, 15).



The numerous papers published in recent years on the rôle of copper carbonate as a seed fungicide should make its use as such familiar to anyone interested in seed treatment. It is used principally to control bunt of wheat and the kernel smuts of sorghum. It also is effective for treating millet, flax, hull-less oats and hull-less barley, although it generally is not recommended for the three latter cereals.

There are two grades of copper carbonate dust disinfectant on the market in the United States. The better grade contains about 50 per cent metallic copper in the form of equal parts of copper carbonate and copper hydroxide. The cheaper grade contains from 18 to 25 per cent metallic copper, mostly as copper carbonate and copper hydroxide, but frequently with some copper sulfate, calcium sulfate, or other ingredients. The better grade usually is sold as "copper carbonate," but dusts made up of the cheaper grade frequently are sold under trade names such as: Cupro-Jabonite, Coppercarb, Smut-Bane, and others.

In general, experience has shown that when seed wheat carries a rather heavy spore load, the better grade of copper carbonate is more effective in bunt control than are the dilute brands, applied at the same rate. When the pure and dilute grades are employed at 2 and 3 ounces per bushel, respectively, they are about equally effective. On the whole, the better grade is preferable both for wheat and for sorghum.

The chief advantages of copper carbonate are its relative cheapness—the cost of treating wheat ranging from 2 to 4 cents per bushel—and its harmlessness to seed germination.

Its disadvantages are its tendency to cause injury to the drill and illness to the operators, and its ineffectiveness under certain soil conditions (115, 116, 128).

Copper sulfate in solution, although still used to a limited extent, is no longer recommended for cereals because it is tedious and disagreeable to apply and frequently is injurious to the seed. As formerly applied and, to some extent, still used, a pound of copper sulfate and a pound of common salt (sodium chloride) are dissolved in from 5 to 10 gallons of water, the grain is immersed in this solution, drained, dipped in milk of lime and then spread out to dry. The milk of lime is made by slaking 1 pound of quicklime (calcium oxide) and then adding enough water to make 10 gallons.

The reaction of the two solutions coats the seed with a basic copper compound that is more or less insoluble and serves to protect the seed, to some extent, from soil infestation.

Copper sulfate in powdered form has been found almost equal in effectiveness to copper carbonate in bunt control (80). Its outstanding disadvantage is its tendency to lose its finely powdered form and consolidate more or less into lumps (80, 129). This prevents it from adhering to the seed sufficiently to be effective as a dust fungicide. If the common pentahydrated form ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) is changed by heat to the monohydrated ( $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ ) or anhydrous form ( $\text{CuSO}_4$ ), it retains its powdered form for a longer time and is more easily applied as a dust disinfectant.

Basic copper sulfate has been found by the writer and by others (115) to be more effective and cheaper in bunt control than most brands of copper carbonate. Unlike copper sulfate (bluestone), it retains its fluffy powdered form about as well as does copper carbonate. It is an especially effective preventive for bunt of wheat, and also may be used for other cereals where copper carbonate has been found satisfactory.

Other compounds of copper have been found effective in the control of certain cereal diseases by the writer and by others (86, 94, 114, 116, 123, 128), but few have reached the stage of profitable commercial production. Copper oxychloride, effective in bunt control, has been marketed but not widely sold in the United States. Copper oxalate, acetate, phosphate, silicate and other less common copper salts have effectively controlled bunt in several years' experiments by the writer, but they possess no particular qualifications that enable them to compete successfully with copper carbonate and other fungicides already firmly established on the market.

Cuprous oxide, now widely sold as a dust fungicide for vegetable seed (47), also was found by the writer to be effective for bunt control but has not been widely used for that purpose.

#### ORGANIC MERCURIALS

There are at present for sale in the United States relatively few fungicides containing organic mercury compounds, although during the past 15 years a considerable number of such materials have been tried in seed treatment investigations (72, 73, 102, 106, 126,

132). Frequently it has been found that the percentage of mercury necessary to make a fungicide effective also makes its cost prohibitive and, therefore, its production unprofitable. The first organic mercury fungicides were used in liquid form. Among these were such products as Chlorophol, Semesan,<sup>2</sup> Seed-O-San, and variously numbered "Bayer," "Corona," "DuPont" and other compounds (126). Along with these were tried various materials of foreign make such as Uspulun, Germisan, Tillantin, and others not commercially available in the United States. With the advent of dust fungicides, experiments with these liquid treatments were largely discontinued and gave way to work on experimental dusts, many of which bore labels similar to those on the earlier products used in solution. Most of these failed to attain commercial success either because of lack of consistent effectiveness, seed injury, or excessive cost. Among the first mercury dusts used to any considerable extent were such materials as—Bayer Dust (hydroxymercurinitrophenol sulphate), Sterocide (mercury furfuramid), Semesan Jr. (hydroxymercuricresol), Semesan (hydroxymercurichlorophenol sulphate), and Merko (3.5 per cent metallic mercury in inert filler).

Some of these materials (Bayer dust, Sterocide) are no longer being made, while others have been considerably changed in composition. Among the better-known mercury dusts now on the market are the "Dubay" products. These are made by the Bayer-Semesan Company, which concern combines the former activities of two other concerns along this line.

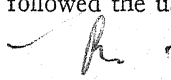
Ceresan,<sup>3</sup> 2 per cent ethyl mercuric chloride, one of the first "Dubay" products, was at first recommended for controlling certain diseases of wheat, oats, barley, sorghum, and flax, and, on the whole, proved effective for that purpose. However, the recommended application of 2 to 3 ounces per bushel made its high cost—9 to 14 cents per bushel of seed—an impediment to its widespread use for cereal-seed treatment. It is now recommended as a treatment for cotton seed (88, 135), peas, and seeds of some ornamentals.

New Improved Ceresan, another "Dubay" product, is now widely used for cereal-seed treatment. It contains 5 per cent ethyl

<sup>2</sup> A product by this name is still being marketed.

<sup>3</sup> The "Ceresan" made in Germany contains phenyl mercuric acetate as its essential ingredient.

mercuric phosphate and is applied to wheat, oats, barley, sorghum, and flax at the rate of only  $\frac{1}{2}$  ounce per bushel at least one day before sowing. It is a so-called volatile dust and much of the disinfection is done before the seed is sown. However, if sown not more than a few days after being treated, the seed carries with it into the soil a certain amount of protection against certain soil-inhabiting organisms that frequently attack germinating seeds and unemerged seedlings. The longer sowing is delayed after treating the seed with New Improved Ceresan the less protection is furnished against these organisms, because more of the volatile fungicidal material will have been dissipated. On the other hand, prolonged post-treatment storage of the seed is most effective against the seed-borne organisms. Under certain conditions, however, prolonged storage may impair slightly the germinative capacity of treated seed.

Another Dubay dust, New Improved Semesan, Jr., is now widely used for treating seed corn. It contains 1 per cent of ethyl mercuric phosphate as its toxic ingredient. Dungan, *et al.*,<sup>4</sup> in extensive field tests with composite lots of seed corn from local farms in Illinois, in 1935, found that this material improved germination and stand, and increased the average yield of sound corn 9 per cent. In the same series of tests the yield from seed treated with Barbak 111 was 3 per cent greater than the yield from untreated seed. Barbak 111 contains 10 per cent mercuric phenyl cyanamide and 5 per cent lead diethyl dithiophosphate. It is advertised solely as a disinfectant for seed corn. Merko, 3.5 per cent metallic mercury in inert material, has compared favorably with other materials in some experiments (44, 84). Excellent results followed the use of these materials in experiments in Iowa (98). 

#### CONTROL OF DISEASES OF VEGETABLES, ORNAMENTALS, AND OTHER CROPS

The control of diseases affecting vegetables and ornamentals is a more complicated matter than that of those affecting cereals because one has to deal with a much greater variety of hosts and parasites. Many of these diseases are soil-borne or spread by wind, insects, or other agencies and do not respond to seed treatment. Methods of control in these cases are restricted largely to soil disinfection, if

<sup>4</sup> Unpublished data.

practicable, crop rotation, sanitation, spraying and dusting or the use of resistant varieties. In growing vegetables and ornamentals a greater variety of cultural practices also is involved. Some seeds, for example, are planted directly in the field, while others are planted in flats or hotbeds and the seedlings later transplanted. The latter method involves operations and problems foreign to cereal culture.

Prevention of damping-off is one of the chief problems in growing vegetables and ornamentals. This trouble is largely caused by species of *Pythium*, *Rhizoctonia*, and *Fusarium*. There are two phases of the disease, the pre-emergence and post-emergence stages. Soil sterilization by steam or by chemicals, particularly in flats or beds for transplants, frequently will control the former but not the latter phase of this disease.

Formaldehyde dust has been widely recommended as a soil disinfectant for damping-off prevention (1, 124, 133) and has met with much success. Recently cuprous oxide applied to the seed has been advocated for the control of damping-off (47) and growers of vegetables and ornamentals have found it very effective, especially for the pre-emergence stage of the disease. Organic mercurials also have been used with some success.

The use of zinc oxide as a soil-surface disinfectant for the prevention of the post-emergence phase of damping-off has met with considerable success (46). In this respect it is not used as a substitute for cuprous oxide but in conjunction with it. It also may be used as a fungicide to combat the earlier phase of the disease but with few exceptions, cuprous oxide is reported to be more effective in this respect (46). However, zinc oxide along with zinc hydroxide incorporated in the proprietary product Vasco 4 was found most effective in Virginia for treating seed of spinach and tomatoes (14, 15). Other materials recommended for soil treatment to combat soil-borne organisms are mercuric chloride solution, calomel, potassium, sodium or calcium nitrate, lime, calcium cyanamide, sulfur, sodium tetraborate, various organic mercurials, acetic acid, ammonium sulfate, and a number of other substances.

The recommendations for controlling seed-borne diseases of vegetables and ornamentals are so many and varied that they will not be reviewed here in detail. For many vegetables, such as the crucifers, tomatoes, onions, and peppers, mercuric chloride still

figures prominently in seed-treatment recommendations. Others specify organic mercurials in liquid or dust form, hot water, calcium hypochlorite, and various other treatments. There are many publications dealing with the general subject of diseases of vegetables and ornamentals and their control (11, 12, 17, 33, 57, 96, 97, 99, 112, 133), in which may be found generally accepted, although not always the most recently developed, methods of seed treatment.

*Forage Crops.*—Seeds of forage crops have not figured very prominently in seed treatment experiments according to the literature on the subject. One objection to the fungicidal treatment of seeds of legumes is its possible effect on the nitrogen-fixing bacteria with which the seeds subsequently may be inoculated (2). Horsfall (45), in his study of meadow-crop diseases and their control, makes no definite seed-treatment recommendations, and there seems to be a paucity of information in the literature upon which to base definite recommendations of that kind. Buckholtz (9) improved seedling stands of alfalfa, *Dalea*, *Lespedeza*, and several clover varieties by treating the seed with an organic mercury dust before sowing in *Pythium*-infested soil, but alfalfa seedling stands were depressed in *Pythium*-free soils.

*Cotton.*—The treatment of cotton seed with fungicides is a comparatively recent development. Delinting with sulfuric acid, while effective in controlling bacterial blight and probably some other seed-borne diseases, is sometimes responsible for poor stands especially in cold wet soil. It also is a somewhat cumbersome and dangerous operation (88). Hydrochloric acid gas (88) also may be used, but treatment with this requires special equipment and is practicable only on a commercial scale. Ceresan (ethyl mercuric chloride) and other fungicidal dusts have been found beneficial by several investigators (3, 135) and are more easily applied than the other two materials.

As the result of 8 years' experiments with fungicides for cotton seed at State College, Mississippi, L. E. Miles<sup>5</sup> recommends Ceresan, iodine dust, cuprous oxide, and zinc oxide as the most promising in their effects on germination and yield. The amount of benefit to be derived, he states, depends greatly upon the condition of the seed—age, viability, and seed-borne organisms—the condition of the soil, and the date of planting.

<sup>5</sup> Correspondence.

*Tobacco*.—Recommendations for treating tobacco seed have not been materially changed during the past 15 years. The control of tobacco diseases is more a matter of soil sterilization, sanitation, and cultural practices than seed treatment (53). Mercuric chloride probably is the most widely used disinfectant for tobacco seed. The seed is soaked for 15 minutes in a 1-1000 solution, then thoroughly washed and dried (26). Immersion in a 1-1000 solution of silver nitrate for 15 minutes (54) followed by repeated washing also is recommended. Formaldehyde (26) also is used but is not as satisfactory because it is somewhat less effective and is more likely to cause seed injury.

Organic mercurials have not been sufficiently tested in tobacco seed treatment experiments to merit recommendation for that purpose.

*Sugar beets*.<sup>6</sup>—Damping-off is one of the most troublesome diseases encountered in growing sugar beets. It may be caused by the seed-borne organism *Phoma betae* and also by certain soil-inhabiting species of *Rhizoctonia*, *Pythium* and *Aphanomyces*. A seed treatment to be wholly effective, therefore, not only would have to disinfect the seed but also would have to protect it and the young seedling from the attack of these soil-borne organisms. Dust fungicides, on the whole, have been found more nearly effective and more practical for this purpose than liquid fungicides, largely because of the residual protection they afford the seed after planting. Borax, copper carbonate, cuprous oxide, formaldehyde dusts and certain proprietary preparations such as Ceresan, Germisan, Agrosan and others have been used with varied results for combatting beet diseases and for improving stands. Other materials have been applied to the soil to protect the seedling during and after emergence. Among these may be mentioned copper sulfate, zinc oxide, sodium nitrate, formaldehyde, cyanide compounds, ammonium sulfate, ammonium hydroxide, boric acid and sodium borate.

The relative effectiveness of a large number of fungicidal materials has been studied in extensive field and greenhouse experi-

<sup>6</sup> The above discussion of beet-seed treatment was prepared with the kind assistance of Dr. G. H. Coons, principal pathologist in the Division of Sugar Plant Investigations. The information was obtained largely from material being prepared for publication as a U. S. Department of Agricultural Technical Bulletin by G. H. Coons, J. E. Kotila, E. L. LeClerc, J. G. Lill, and S. B. Nuckols.

ments carried on by workers in the United States Department of Agriculture for a number of years. The most effective fungicidal dusts developed by these workers contain both mercuric chloride and copper carbonate, the latter serving both as a diluent and as a slowly soluble or residual fungicide. Strikingly beneficial results have been obtained in greenhouse and small field trials, but large-scale tests have revealed a very complex situation which needs further study and analysis before the treatment of sugar-beet seed can take its place as a routine farm practice. The results of beet-seed treatment are influenced not only by environmental conditions but also by the type of pathogen predominating in the soil. The preceding crop also may be an important factor. Certain crops seem to augment the number of pathogenic soil organisms; other crops apparently repress them. With the aid of a rotation system employing desirable crop sequences, and the improvement of soil conditions by drainage and the application of fertilizers, it is very likely that the proper treatment of sugar-beet seed will result in securing satisfactory stands under all but the most unfavorable conditions.

#### CENTRALIZED SEED TREATMENT

In the earlier stages of its development, seed treatment was entirely a farm operation carried out on the farm by the farmer. The increase in the loose smuts of wheat and barley in certain sections of the country shortly after the war created a demand for seed that had been subjected to the hot-water treatment. Since this treatment is not easily carried out on the average farm, central treating stations were established in certain localities by energetic county agents and others, and these proved very successful (95). This lesson in centralized seed treatment was remembered after dust treatments became popular, and custom treating has become the practice in many localities. Millers or elevator operators who have installed efficient large-scale treating outfits charge from 3 to 6 cents per bushel for treating the farmers' seed grain. The disadvantage of this arrangement is the trouble involved in hauling the grain to and from the central treating unit.

This objection to custom treating is overcome by the use of portable or itinerant cleaning and treating outfits. These are compactly made and mounted on trucks. They go from farm to farm,



thus saving the farmer the trouble of hauling his seed to a central plant. The charge is about 4 to 8 cents per bushel for cleaning and  $2\frac{1}{2}$  to 5 cents for treating. Outstanding examples of this type of outfit are Keck Gonnerman "Kay Gee" cleaner-treaters operated in Indiana, and the "Discreenair" service in California.

Some seedsmen are making it a practice to treat seed of wheat, oats, and barley before they sell it to the farmers. T. W. Wood and Sons, of Richmond, Va., The Eastern States Farmers' Exchange, Springfield, Mass., and the Grange League Federation, Buffalo, N. Y., are pioneers in this direction. The Richmond concern at first restricted its activities in this field to dusting wheat with copper carbonate at the request of the customer. All wheat, oats, and barley now sold by them to the retail seed trade is first treated with ethyl mercuric phosphate (New Improved Ceresan). Several seedsmen in New York apply the dry formaldehyde treatment to seed oats for sale. Others use organic mercury dusts. Inquiries regarding approved materials and equipment for treating seed on a large scale seem to indicate a growing interest on the part of seedsmen in selling treated seed. The problem of efficiently applying the different dust fungicides to cereal seeds has been met by the development of a number of improved devices for this purpose (50).

#### EFFECTS OF SEED TREATMENT

Plant stimulation from seed treatment is a subject that has received considerable investigation. It generally has been shown that any benefits derived from seed treatment can be traced to the control of organisms found either on or in the seed or in the seed bed. Occasionally, when untreated seed is sown for comparison, pronounced differences in vigor, stand, or yield are noted without any apparent indication of disease in the plants from untreated seed. These differences are then likely to be regarded as indications of stimulation. The diseases prevented or controlled may not, however, be externally evident because of lack of sporulation, *i.e.*, so-called latent infection may be present in the plant (138, 139). This has been shown to occur especially in resistant varieties. Flor *et al.* (24) demonstrated it in the case of bunt, Hubbard and Stanton (48) in oat smut, and Zade (138, 139) in several cereal diseases. Others have shown that little or no benefit was

derived from treatment of sound disease-free corn, planted under favorable soil moisture and temperature conditions (58, 59, 81, 82, 108, 109, 110). Similar results have been obtained with oats (74). On the other hand, when germination is delayed by cold, wet soil fungicidal dusts may protect the seeds and seedlings from attack by soil organisms which otherwise might attack and destroy the seed itself or invade the slowly developing seedling and either kill it or cause a weak plant of low yielding capacity.

With the extensive amount of research being conducted by commercial concerns and also by State and Federal agencies, on the development of disinfectants for the control of plant diseases, the composition of fungicides will continue to change. Materials now being widely used will either be further improved or will be replaced by other materials that will be more effective, cheaper, less harmful to the seed, or more acceptable in other respects. The constant aim will be to find or develop disinfectants that are highly toxic to parasitic fungi and bacteria but relatively harmless to the seeds and plants parasitized by them.

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# THE BOTANICAL REVIEW

VOL. II

NOVEMBER, 1936

No. 11

## CHROMOSOME STRUCTURE IN RELATION TO THE CHROMOSOME CYCLE

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Present conceptions of chromosome structure have resulted from a critical appraisal of diverse cytological interpretations in the light of genetic theory, for it has become increasingly evident that maintenance of the linear order of the genes through the mitoses depends on some permanent constituent of the chromosome. Such structure has been observed in several species of plants and animals as a slender coiled chromatic thread, the chromonema. Recognition that the chromonema carries the gene string, or genomema, has permitted correlation of the genetical and cytological findings.

Chromonemata were first discovered in 1880 by Baranetzky (2) as spiral bands in chromosomes of living sporocytes which he had pressed from anthers of plants of the genus *Tradescantia*. His contemporaries and immediate successors, however, derived other interpretations of chromosome structure, primarily from fixed material. In the early part of the present century chromonemata were described anew in such studies as those of Bonnevie (8, 9) and Vejdovský (114). Nearly all the investigators of that period regarded the chromonema as transitory in the mitotic cycle. About ten years ago Kaufmann (41) reported that the chromosomes of *Tradescantia* contain double spiral bands throughout somatic and meiotic divisions. Proof of the permanency of the chromonema has accumulated during the past decade, following the refinement of technical methods and the application of special procedures, and will be considered in the present article.

### SOMATIC MITOSIS

*The Structure of the Chromosome.* For the study of somatic mitosis, plant cytologists have relied primarily on paraffin-imbedded

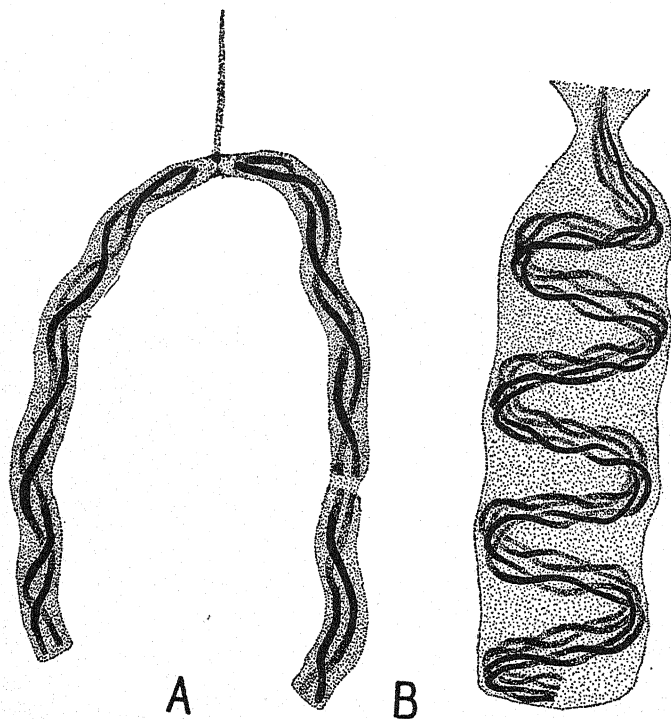


FIGURE 1A.—Diagram of somatic anaphase chromosome, with sub-median spindle-attachment region showing attachment chromomeres, and with secondary constriction in the longer arm. The chromomeres are represented as intertwined and embedded in a common achromatic matrix.

FIGURE 1B.—Diagram of one homologue of the *Tradescantia* type of bivalent at metaphase of the first meiotic division. Sister chromatids lie in close contact, and are coiled as major and minor spirals. Each chromatid is represented as consisting of a pair of intertwined chromonemata. All are embedded in the achromatic matrix.

and sectioned material. In such preparations the cylindrical metaphase and anaphase chromosomes often stain deeply and uniformly, except at the constrictions. These are relatively slender, achromatic regions, which are constant in position in a given chromosome (fig. 1A). Each chromosome has a spindle-attachment constriction, and may have one or more secondary constrictions. Within the attachment constriction, as is seen best in chromosomes with non-terminal spindle-attachment, are the minute granules, or "kinetic bodies", from which the "spindle fibers" appear to extend.

The arms of deeply stained chromosomes sometimes exhibit a moniliform contour which furnishes an intimation of the presence of chromonemata. Resolution of the spiral filaments is aided by such methods as prefixation (95, 77), freezing (21, 23, 24, 25) and differential staining. That the chromonemata exist in association with another substance, designated as the matrix, has been inferred not only from the direct observation of fixed and living chromosomes, but also from histochemical tests, which suggest that the spiral portion of the chromosome consists chiefly of nucleoproteins, the matrix mainly of lipoids (100, 97).

The chromonema, usually observed as a smooth thread of relatively uniform diameter, may appear at times as a linear aggregate of small chromatic particles, or chromomeres. They are seen most clearly when the chromonema is extended, as in the early prophases, and probably lie close together when the chromosome is contracted. Chromomeres are more pronounced in some organisms than in others, but when clearly defined they show a specificity of size and constancy of position which testify to their reality. Such true chromomeres, which present morphological evidence of the linear differentiation of the chromonema, are not to be confused with the chromomere-like aspect of delicately coiled threads (43, 27), nor with the dots and bands arising from the refraction pattern of a spiral (80).

*The Number of Chromonemata.* Despite the frequent observations of chromonemata, the number per chromosome at the different stages of mitosis remains a matter of controversy. The anaphase chromosome, for example, has been regarded by different investigators as composed of one, two or four chromonemata. Nor are these divergent opinions to be attributed solely to dissimilarity of the chromosomes of different genera,<sup>1</sup> since they have followed studies of chromosomes of the same genus. Substantial support has been given the interpretation that the anaphase chromosome contains two more or less intertwined chromonemata (42, 43, 95, 108-111, 26, 85, 29, 101, 48, 27), which persist through the telo-

<sup>1</sup> Nebel (82) suggests that the number of chromonemata may not be the same for all organisms. In the Diptera, the salivary gland chromosomes which undergo no further division have been interpreted as containing several chromonemata, which presumably arise from the original one without attendant chromosome division. Koltzoff (46) and Bridges (10) report that there are 16 chromonemata per "somatic bivalent" in *Drosophila melanogaster*; Bauer (3) reports 100-400 in certain Chironomidae.

phase and interphase to become the sister chromatids of the prophase chromosome. Each of these chromatids reveals a new split in late prophase or early metaphase to form the four chromonemata of the metaphase chromosome (42, 43, 108-111, 26, 85, 29, 101, 48, 27, 34, 35, 21).

The validity of certain other interpretations has been demonstrated less convincingly. According to Nebel (77, 79, 80, 82) and Stebbins (102) the quadripartite condition is recognizable in the telophase, and Nebel has suggested that each chromosome has four chromonemata throughout mitosis, although only two may be visible at anaphase. Recently, Goodspeed, Uber, and Avery (25), employing the Altmann freezing-drying technique have reported that the anaphase chromosome of *Lilium* contains four visible chromonemata, and the metaphase chromosome an 8-partite chromonematic complex.

At times only a single chromonema can be detected in anaphase and telophase chromosomes. This unitary condition often has been ascribed to lateral approximation of two or more chromonemata obliterating evidence of their individuality (42, 95, 29, 27). The explanation also has been offered that singleness at these stages represents the normal condition (13, 15, 16, 7) and that suggestions of multiple chromonemata follow either fixation artifact or cytological misinterpretation. Thus, Belling (7) contended that the appearance of duality in fixed material represents an artifact, since living resting nuclei contain unsplit threads, but this view is contested by the observations of Telezyński (109) and Kuwada and Nakamura (58) of twin chromonemata in living telophase chromosomes of staminate hairs of *Tradescantia*. Darlington's assumption that the aspect of duality rests on misinterpretation of optical sections of chromosomes disregards the contrary evidence of widely diverging chromosome ends, split satellites (106, 21) and end views to chromonemata. Objections of a decade or more ago to anaphasic duality (60, 50) attendant on the then current parasynapsis-telosynapsis controversy, were not concerned with the number of chromonemata, *per se*, but rather with the cleavage of the entire chromosome, which, it was held, would traverse the turns of the coils and disrupt the linear order of the genes (*cf.* 13, 16).

Treatment of chromosomes with x-rays has been employed in an effort to test the validity of these divergent interpretations of the descriptive cytologists. The experiments have proceeded on the assumption that if the chromonema is unsplit at the time of radiation, any alteration produced will be transmitted with the subsequent division of the chromosome equally to the daughter chromatids. On the other hand, unequal abnormalities would be expected from divided chromonemata, since each thread would be affected independently at the time of treatment. Mather and Stone (71) detected only chromosome breaks or equal abnormalities following irradiation of corms of *Crocus*, and concluded that the chromosomes are not split prior to the resting stage, when the rays apparently initiate the abnormalities (105). Failure to discover chromatid breaks is not sufficient proof, however, that the chromonema is undivided. Sax and Sax (93) note that the experiments conducted by Riley indicate that the split chromosome of *Tradescantia* behaves as a unit in response to x-ray treatment, since the microspore nuclei rayed during the resting stage show only chromosome breaks at metaphase. As Huskins and Hunter (33) have indicated, the further possibility exists that the broken ends of the chromatid may rejoin and thereby eliminate evidence of the alteration. Moreover, if radiation can affect either chromonema or matrix, as Mather (70) and Moore (76) have suggested, chromosome breaks may result from severing of the matrix and all the contained chromonemata, so that their subsequent behavior resembles that of an undivided thread.

By way of contrast, chromatid breaks furnish strong evidence that the chromosome is double at the time of irradiation. One such break was found by Lewitsky and Araratian (61) in x-rayed *Crepis capillaris*. White (116) detected both chromosome and chromatid breaks in spermatogonia of *Locusta migratoria* following irradiation of newly emerged males, although in his direct observation no trace of an anaphase split was encountered (contrast 75, 87). Huskins and Hunter (33), staining the chromosomes of *Trillium* to detect chromatid as well as chromosome breaks, found both types following irradiation of anthers containing cells in the second meiotic telophase or in the early resting stage. Since telophasic duality has been detected cytologically in root-tip chromosomes of *Trillium* (95, 34), the most convincing evidence from

radiation experiments indicates the existence of at least twin chromonemata during anaphases and telophases.

*The Telophases: The Rôle of the Matrix.* Chromonemata of stained preparations are differentiated more readily during the telophases than during the anaphases. Frequently this has been attributed to the loss of chromaticity or to the alteration of the matrix substance. Some observers have suggested that the matrix becomes continuous with the karyolymph of the newly formed nucleus, the chromonemata alone maintaining genetic continuity during the interphases (42, 95, 29, 27); others that the matrix remains associated with the coiled threads (4, 77, 79). Of the latter group Nebel holds the singular opinion that each spiral thread is sheathed in its own thick matrix, that under conditions of fixation the matrices may become confluent to simulate an investing material common to all the chromonemata. Then there is the theory that the matrix substance contributes to the formation of the nucleoli (see 96 for lit. cit., 64, 20, 74) which are developing synchronously with the skeletonization of the chromosomes. In this connection, McClintock (74) has reported that when the nucleolus-forming region of chromosome 6 of *Zea mays* is absent, or when its activity is impaired by certain chromosome deficiencies, the nucleus does not develop the large nucleoli typical of normal strains of *Zea*, but many small nucleolus-like bodies. These appear at indefinite positions along the chromosomes, apparently by the collection of droplets of the matrix substance. The evidence that the nucleoli form from the matrix is not conclusive, but "it is difficult to avoid the impression that a distinct relationship exists between the two" (74). Another activity attributed to the matrix is the formation of anastomoses (26, 74) which appear in many preparations as processes arising from the chromosomes and bridging the distances between them. Such outgrowths have been regarded more frequently as derivatives of the chromonemata (42, 43, 109, 111, 101, 29, 27, 25) or even as artifacts because of their variability or absence in certain preparations (79, 48).

Apart from explanations involving alterations of a matrix material in the anaphase-telophase transformations is the interpretation that loosening or extension of the coils suffices to render them more conspicuous. Darlington (15) has denied the existence of a matrix substance which occupies the spaces between the turns



of the coils, and Upcott (113) in support of this contention has presented an unconvincing analysis purporting to show that the entire volume of each chromatid is occupied by a tightly coiled chromatin thread.

When multiple chromonemata have been recognized in the anaphase or telophase chromosome they usually appear to be intertwined. It has been suggested, however, that the apparent intertwining may represent close approximation of independently coiled threads (25). In support of the latter alternative, Nebel (77, 79, 80) has made a comparative study of models of chromosomes composed of translucent materials, which he believes permits a more accurate appraisal of the microscopical images of chromonemata. His contention that the chromonemata of *Tradescantia reflexa* do not entangle at any stage is at variance, however, with the numerous observations of intertwined prophase and metaphase chromatids of *Tradescantia* as well as of other plant genera.

*The Interphases; Continuity of the Chromonemata.* As the irregularly shaped early telophase nucleus enlarges to assume a spherical form, the chromosomes become extended, their coils loosened. While retaining the telophasic arrangement at the proximal or spindle-fiber-attachment region, the arms of the chromosome lose their more or less straight arrangement, and are distorted into large loops or zigzags, which have been designated as superspirals (16). Following this period of extension and attenuation the interphases are reached. The general appearance of the interphase nucleus, apart from the nucleoli, is of a network or reticulum, which is composed, according to most interpretations, of the chromonemata and interchromosomal processes. In some organisms, however, portions of certain chromosomes, often adjacent to the region of spindle-attachment, remain condensed and deeply chromatic (heteropyknotic) instead of contributing to the formation of the reticulum.

This period of transition from telophase to prophase presents the greatest obstacle to cytological verification of continuity of chromonemata. Darlington (13) has maintained that all direct evidence of structure in the resting nucleus is unreliable, but there seems little basis for doubt that the granules and rods so frequently observed in fixed material represent optical sections of chromonemata, as Martens (67, 68), Bělař (4) and Teleżyński



(108, 109) found in living nuclei. In addition, less direct types of evidence of continuity are available (see 117, 4, 96 for lit. cit.). For example, the abnormalities produced following x-ray treatment of metabolic or resting nuclei furnish cogent evidence, as Sharp (96) has indicated, that even at these stages the linear organization of certain elements of the chromosome is normally maintained.

*The Prophases.* With the onset of the prophases, the reticulum-like aspect disappears; the chromosomes become more pronounced and are distinctly split longitudinally. To proponents of the theory of the undivided telophase chromosome, this represents the initial appearance of the split, and suggests that division occurs during the interphases (13, 16). Sister chromatids seem to be twisted about each other, but as the prophases advance and the chromosomes shorten and thicken, the amount of twisting decreases. An occasional wide separation of the chromatids during the middle prophases has been reported (95), although intertwining to some degree usually persists until metaphase (42, 109, 111, 101, 29, 85, 48, 27, 16). Meanwhile, along the chromatids, new coils become pronounced. Their doubleness and the consequent quadripartite nature of each chromosome may not be evident until late prophase or early metaphase, although actual division apparently occurs much earlier, the split being obscured by the close approximation in pairs of the half-chromatids.

Such questions as when and how the chromonema divides, how spiralling and uncoiling occur, will be considered later.

#### MEIOSIS

*Structure of the Bivalent.* Knowledge of the behavior of chromonemata during meiosis has accumulated primarily from the study of microsporocytes of plants with large chromosomes. The ease with which such cells may be expressed from the anther has permitted a wide array of observations both on living and treated material (see 96 for lit. cit.). Most frequently studied have been the compact bivalents of first metaphase. It will be recalled that such chromosomes of *Tradescantia* served as material for Baranetzky's original observation of the chromonema. Subsequently it was recognized that each of the spirals which he described represents a pair of closely appressed chromatids (42), the bivalent being a tetrad. In other genera, as in *Gasteria* (107), *Trillium*

(35) and *Fritillaria* (16), the tetrad structure is more readily discernible, since the paired chromatids are less intimately associated. The helical course of the chromatids defines the cylindrical form of the chromosome seen in the average sectioned and deeply stained preparation. End views, accordingly, appear as rings with the denser staining, or, if living, with the more refractive material peripherally disposed (11). In well-flattened smears a membrane or sheath may be identified, delimiting the chromosome and often removed some distance from the chromonemata (42, 35). Because of such evidence many observers have concluded that the axial region of the chromosome and the spaces between the turns of the coils is occupied by an achromatic matrix substance.

Such wide spirals as have just been considered may be designated as major spirals since recent observations have shown that they in turn consist of compact minor spirals. First recognized by Fujii (22), this spiral-along-the-spiral type of organization has been verified extensively; in *Tradescantia* (22, 37, 52, 54, 55, 57, 39, 92), in *Hosta* (37), in *Sagittaria* (99), in *Lilium* (99, 40), in *Trillium* (73), in *Fritillaria* (16). When the major spirals are unraveled artificially with ammonia vapor, presumably by removal of the matrix material, as Kuwada and Nakamura (55) demonstrated, the chromatids with their minor spirals present an aspect resembling a nucleus in interkinesis or interphase, except that there are no nucleoli.

Another recent advance in our knowledge of the structure of the metaphase bivalent has been the identification of eight chromonemata, resulting from the longitudinal splitting of each of the four chromatids (fig. 1 B). Observations of this "tertiary split" by Nebel (77), Huskins (30), Shinke (99), Iwata (reported by Kuwada, 53), and Huskins and Smith (35)<sup>2</sup> have been supported by certain radiation experiments. Thus, Marshak (65, 66) noted attached chromosomes at first anaphase in *Gasteria* which could be explained by the occurrence of a chromonematic division following irradiation at a four-strand stage. Moore's study of irradiation-produced mutations in the vinegar-fly, *Drosophila melanogaster* (76), indicates that each of the four chromatids at first meiotic metaphase in the egg contains two sets of genes in

<sup>2</sup>Also personal communications of Dr. Barbara McClintock and Dr. H. Dermen.

chromonemata or potential chromonemata, as do likewise the chromosomes of the mature sperm. Patterson (84) concluded, however, from a study of the effects of x-radiation on the production of mosaic flies by breaks in the X-chromosome, that this chromosome is split in about one out of every seven sperms. Moore has attempted to reconcile this conclusion with his findings by suggesting that chromosome breaks may represent a phenomenon of the matrix, so that "a break of an undivided matrix results in the severing of all the chromonemata, the behavior in subsequent inheritance suggesting a single chromatin thread."

*The Leptotene Threads.* To trace the origin of the eight chromonemata it is necessary to consider the prophases of the first meiotic division. Leptotene chromosomes frequently appear as single threads. This condition could result from failure of the chromosomes to split in the last pre-meiotic mitosis (30). There is, however, considerable evidence contrary to this view, since Smith (101), Koshy (47) and Hoare (27) report that the last pre-meiotic division of various plants does not differ from preceding mitoses with respect to the time of splitting of the chromonemata. In the Orthoptera, McClung (75) and Robertson (87) find the telophase chromosomes longitudinally split in the last spermatogonial division, which can be identified accurately. Moreover, a few observations of duality in leptotene threads have been recorded, those of Kaufmann (44) on *Tradescantia* and *Rhoeo*, of Koshy (49) on *Allium*, of Huskins and Hearne (32) on asynaptic oats and wheat, of Huskins and Smith (34) in portions of the chromosomes of *Fritillaria Meleagris*.<sup>3</sup> The latter authors have found in *Trillium*, however, unsplit leptotene threads, and have attempted to reconcile these divergences by suggesting that the split occurs in those portions of the chromosomes which will not pair, the underlying theory being that synaptic attraction exists only between single threads (12, 30). Whatever the merits of such a theory when applied to forms in which pairing is incomplete, as in *Fritillaria*, asynaptic plants, and the interchange heterozygotes of *Tradescantia* and *Rhoeo*, it fails to explain the complete pairing of divided chromosomes, both meiotic (49) and somatic.

<sup>3</sup> Lorbeer (Jahr. Wiss. Bot. 80: 567-818. 1934) reports that among the liverworts, the leptotene threads of *Sphaerocarpos Donnellii* are clearly double.

Best demonstrated in the Diptera, the phenomenon of somatic pairing has been studied carefully in *Drosophila melanogaster*. Anaphasic duality and prophase conjugation of split chromosomes with the resulting chiasma-like configurations have been detected cytologically (45), and there is genetic evidence of somatic crossing-over which occurs at a four-strand stage (103, 104).

Another explanation of the optically single leptotene chromosome involves the closing or "healing" of the pre-meiotic split. This seems a probable corollary of the attenuation and extension of the chromosomes at these stages. Inability to differentiate optically the intimately associated threads of fixed preparations is a matter of common cytological experience, and more recently Huskins and Smith (35) have recognized that doubleness or singleness may be a physiological and reversible state.

Leptotene threads of many organisms present the appearance of delicate beaded threads, because of the deeply staining chromomeres, specific in size and occupying corresponding positions in the homologues (see 86, 96, 94 for lit. cit.). In certain cases, chromomere-like aspects have been interpreted as effects produced by intertwining of sister chromonemata or by tight coiling (63, 44, 49). Koshy (49) reports that the two intertwined chromonemata become independent coils before synapsis.

*Synapsis to Metaphase.* Synaptic association often begins at the attachment constriction or at the ends of the chromosomes. Homologous chromomeres pair side by side to form the pachytene chromosome. According to Darlington (17), the parts of the chromosome which pair first condense first, as evidenced by the behavior of the proximal or spindle-fiber-attachment region of the chromosomes of *Fritillaria*. Pachytene chromosomes frequently are 7 to 11 or more times as long as at first metaphase (93, 66), and a certain amount of linear contraction may occur prior to coiling, as Belling (5, 6) noted in *Lilium*. During or at the end of pachytene, the equational split between sister chromatids becomes conspicuous, the chromosome presenting then a four-strand appearance. Diplotene begins with the separation of the paired chromatids, which reveals the chiasmata and whatever intertwining exists between the homologues. Huskins and Smith (35) and Darlington (18) have interpreted their preparations of these stages in *Trillium* and *Fritillaria*, respectively, as demonstrating that

separation on the two sides of a chiasma is always "reductional," that is, between homologues rather than sister chromatids, the chiasma resulting, therefore, from an exchange between two chromatids of partner chromosomes (the one-plane theory). Such accurate identification is extremely difficult in most plant material, although extensive studies of this kind have been made on the Orthoptera, where, according to McClung and his students, opening out of the chromatids occurs along the equational as well as the synaptic plane (the two-plane theory).

As diplotene progresses, the chromosomes shorten, thicken, and assume a moniliform contour. Evidently spirals are developing, but their relation to the major and minor spirals of metaphase is less certain. Darlington (18) interprets mid-diplotene spirals of *Fritillaria* as minor ones. They are presumably complete by late diplotene, whereas the major spirals are first discernible at that stage, and are not complete until diakinesis. According to the interpretation of Kuwada and Nakamura (54, 53), the major spirals of *Tradescantia* are established first, the minor spirals resulting from the secondary coiling of the strand which forms the spiral. Sax and Sax (93) state that the minor spirals appear to begin development before the major ones, but that they may develop so slowly that they continue to coil, or at least contract, after the major spirals are established in early metaphase. In *Trillium erectum*, Huskins and Smith (35) find that the major spiral is established in mid-late diakinesis. No evidence of a compact minor spiral was observed, although the coiled chromatid twists on its axis and is loosely waved somewhat like the minor spiral of Nebel (77). In *T. kamtschaticum*, however, both the major and minor spirals have been observed by Matsuura (73).

At diakinesis the strongly contracted chromatids are spiralled in pairs except where they change partners at the chiasmata. Resolution of the chiasmata may not occur until anaphase, as in *Trillium* (35), although in many species the dyads are connected at metaphase only by their ends. The significance of the matrix in maintaining such pairing has been emphasized by Sax and Humphrey (92), Huskins and Smith (35) and Sax (91). The number of major spirals seems constant in a given homologue at metaphase (90, 107, 78, 39). Matsuura (73) reports that in *Trillium kamtschaticum* the pitch of the coils is the same in all of the five

bivalents, and concludes that the length of the chromosome is a function of the number of spiral gyres. During late metaphase the closely appressed chromatids of the *Tradescantia* type of dyad become more widely separated, without entangling or extension of the major coils. In *Secale*, however, the major spirals tend to straighten out before the paired chromatids separate in late metaphase (90). Since this occurs without elongation of the meiotic chromosome, the drawing-out of the major coils has been attributed to the development of the minor spirals at this stage (93).

*Anaphase I.*—In early anaphase as the proximal regions of the dyads move apart, the distal remaining in contact, the major coils may be pulled out temporarily, but with the release of tension the spirals again contract. The direction of coiling, especially clear at these stages, is evidently not a stable or genetic character (91, 81, 82). Sister chromatids of *Lilium* with terminal spindle-attachment may both coil in the same direction, either dextrorsely or sinistrorsely; they may coil in opposite directions; or, the direction of coiling may change along one or both of the chromatids. Iwata (38), in making this analysis, found that the four classes occur with about equal frequency. In chromosomes with non-terminal spindle-attachment, coiling is frequently in the same direction in both arms (90, 78, 92). When the direction of coiling reverses, it is usually at the spindle-attachment region, occasionally along the arms (90, 107, 38, 35, 91). Such changes between spindle-fiber and distal end occur in *Trillium* almost invariably in equivalent positions in two of the four chromatids, and are about twice as numerous as the chiasmata at diakinesis or metaphase. Therefore, Huskins and Smith (35) have noted that such changes of direction, associated with chiasmata, are related, though probably only facultatively, to crossing-over. To test the relationship between coiling and the mode of crossing-over, Nebel and Ruttle (83) have studied the direction of coiling at first metaphase and anaphase in sporocytes of *Tradescantia reflexa*. They interpret their observations as favoring the two-plane theory.

*The Second Meiotic Division.* At late anaphase the paired chromatids, closely associated only at the spindle-fiber region, form characteristic V- or cross-shaped figures. Following their movements to the poles the strongly contracted chromosomes may pass directly to metaphase of the second division, without material

change in structure, as in *Trillium* or in the abnormal *Gasteria* studied by Tuan (112), but more commonly nucleus formation intervenes. Loosening of the major coils attends the extension and adjustments of the chromosomes in the enlarging nucleus, but the degree of interkinetic uncoiling may vary in different plants, or even in different chromosomes. At second metaphase the chromosomes may show only minor coils, as in *Tradescantia* and *Rhoeo* (92, 39, 91, 93), or both major and minor coils, as in *Sagittaria* (99) and *Fritillaria* (16). In certain species of *Lilium* (40) and *Vicia* (93) some chromosomes may show major and minor spirals, others only minor ones.

The anaphase or telophase chromosomes of the second meiotic division have been described as two-parted in *Allium* (48), *Gasteria* (107), *Galtonia* (101), *Rhoeo* (91), *Scilla* (27), *Tradescantia* (58) and *Trillium* (35). Kuwada and Nakamura (58) find that these paired chromonemata of *Tradescantia* are not twisted about each other; they have been described as intertwined in *Allium* (48), *Galtonia* (101) and *Scilla* (27). The anaphase-telophase split of the second division between chromonemata which are to separate at anaphase of the first postmeiotic division is probably referable to the "tertiary split" of the first meiotic division, but is sometimes not evident prior to the prophases of the second division (107, 101, 48, 27).

*First Postmeiotic Division.* There have been but few studies of chromosome structure during the first division in the microspore. In *Fritillaria*, according to Darlington, the prophase resembles a somatic prophase except that the superspirals are more clearly developed. In *Trillium*, Huskins and Smith find that the metaphase chromosomes are quadripartite, the anaphase chromosomes double. In *Tradescantia*, Sax and Sax find some evidence that the metaphase and anaphase chromatids contain two threads which are coiled together.

#### CHROMOSOME MECHANICS

*Spiralization.* Chromonemata usually are observed as coiled threads. Apparently somatic chromosomes are not devoid of coils at any phase of mitosis. In meiosis, apart from the chromomeric aspect of leptotene and early postsynaptic stages, coiled chromonemata are conspicuous throughout the first and second divisions.



In the sequence of mitoses it seems, therefore, that the old coiling is reduced and disappears as the new coiling comes to completion. Accordingly, it has been suggested that the new spirals aid in drawing out the coils persisting from the previous division (56, 58, 93). This process is related to the fact that in general the maximum number of turns of a spiral obtainable in a thread depends on its length and thickness. When the thread is shortened and thickened, the number of coils is reduced. In a double-stranded spiral the number of twists between the strands is equal to the number of turns of the spiral, and will be reduced as the strands shorten and thicken. Thus in the somatic prophase new coiling causes shortening and thickening of the chromonemata, with the gradual reduction of the old coiling and of the twisting residual from the preceding telophase (58). Kuwada (53) suggests that twisting of the chromonema leads to coiling and seems to be determined primarily by an internal factor, with the contraction of the matrix playing an accessory rôle. Comparison is made between chromonema coils and those of the tendril which are due to internal twisting, and which show the types of coiling with respect to direction observed in chromosomes. The interlaced condition of anaphase and telophase chromonemata is regarded as supporting evidence for the hypothesis of coiling by internal twisting, the assumption being made that the half chromatids coil together during the prophase. Concerning the probable method of formation of the double-coiled, double spiral of the first meiotic division, the explanation is offered that early prophase coiling draws out the old or residual spirals, that each chromatid coils independently so that the two major spirals do not interlace, and that as the minor spirals are established, the major ones are not drawn out because of the contracting force of the matrix (54). To this secondary coiling is attributed, however, the untwisting of the two intertwined chromonemata of each of the four chromatids of first prophase in such plants as *Tradescantia reflexa*, in which the chromosomes of second metaphase and anaphase show parallel but independent spirals (58). The occurrence of intertwining chromonemata in other plants at second anaphase is ascribed to division or separation of the half-chromatids at a later period in the first division than occurs in *Tradescantia*, possibly after the secondary coiling has been completed (58).



*Splitting of the Chromonema.* A problem related to coiling is that of the time and method of splitting of the chromonema. As indicated in the first section of this paper, there are various interpretations concerning the period at which the split first becomes visible. In some plants this seems to be at about the time that spiralling begins (35, 93). Sax and Sax (93) are of the opinion that the split occurring in each of the chromatids during mitotic prophase causes them to coil independently, whereby the remnant coils of the preceding anaphase are removed. Huskins and Smith (35) find that in both mitosis and meiosis the spiralling begins more or less coincidently with the first appearance of the split in the chromatids, and have presented their "heterogonic growth" theory of spiralization. They have accepted Kuwada's earlier explanation of the pattern of coiling, namely, that for each turn of the spiral there is a twist of the two threads about each other in the opposite direction, so that the two coiled threads may separate, without entangling or uncoiling, as occurs in the dyad of *Tradescantia* at late first metaphase or at anaphase (51). They have observed that the half-chromatids of *Trillium* are twisted about each other shortly after the appearance of the "tertiary split" of the first meiotic division. If growth in thickness of the half-chromatids then occurs during the metaphase and anaphase on the outer side of the chromatid, it will occur spirally around them with a reversal of direction once in each gyre of the chromatid spiral, and will be on opposite sides of each half-chromatid in each successive gyre. The heterogonic growth, together with the tension produced by syneresis, will provide a self-perpetuating mechanism for spiralization. Sax (91) has pointed out, however, that the transition from about five major spirals of the first meiotic division to the 20-25 minor spirals of the second division in *Tradescantia*, and comparable behavior in *Rhoeo*, is difficult to reconcile with this hypothesis.

Kuwada and Nakamura refer the split in each chromatid to the interphase, and note that if the halves are still intimately associated when coiling begins in the prophase, they will twist about each other, but should they separate and coil independently no aspect of interlacing will be presented. Darlington (15) likewise holds that splitting occurs at interphase, although one mitosis later than that postulated by Kuwada and Nakamura. On the contrary, the

8-partite metaphase chromosomes of *Lilium* observed by Goodspeed, Uber, and Avery suggest that division may occur approximately three mitotic cycles before separation is accomplished. These investigators conceive of gene division as occurring at the end of the resting stage, with the succeeding prophase and early metaphase devoted to an elaboration of a visible chromonema.

Since in somatic mitosis the chromosomes do not appear free of coils at any period, full extension of the chromonema apparently is not prerequisite to its longitudinal division. A plane of division in a coiled thread, which follows the turns of the spiral, will lead to intertwined halves (*cf.* 48, 27). Darlington (18) suggests that division involves the splitting of one large thread into two equal small ones in such a way that the two if straightened without rotation of the ends would lie in one Euclidean plane. Then there is Nebel's contention that the chromonemata do not entangle at any period. To account for this condition he has suggested that regardless of the time of reduplication, if it occurs in only one plane, passing through the main axis of the chromosome, no difficulty will arise in separating the daughter chromonemata even if they are coiled spirally (81).

*Coiling and Crossing Over; An Hypothesis.* The analysis of the internal mechanics of chromosomes presented recently by Darlington (16-19) involves his interpretation of the various types of coils observed during mitosis and meiosis in *Fritillaria*. The property of the formation of spirals is attributed to an internal twist due to a rearrangement of the constituent particles, either between molecules, or within molecules. This molecular spiral is a compensating twist and leads the thread to coil in an internal spiral in the opposite direction to that of the major and minor spirals. The direction of the molecular spiral is subject to unitary control in each of the arms of the chromosome. In somatic mitoses the anaphase and telophase spirals do not relax completely prior to the resting stage when division into paired chromatids occurs. The spirals are uncoiled gradually during the prophases, and as a result the two chromatids become twisted about each other in the opposite direction (the so-called relational spiral). In meiosis the prophase begins precociously, before the chromosomes have divided and before they have reached their maximum extension. Consequently, the two homologues coil round one another to compensate for any

uncoiling which has survived the leptotene stage. As the molecular spiral continues to uncoil during pachytene beyond what is necessary to straighten the chromosomes, there is produced a system in which, when the chromosomes divide, they will be twisted around one another in the opposite direction to that in which they are coiled internally. The four chromatids are therefore in a state of tension resulting from the conflict of lateral attraction and longitudinal cohesion. This state of tension may be relieved by breakage of one of the two chromatids of each homologue at corresponding levels. Genetically this leads to crossing-over; the union of the broken ends to a recombination. Crossing-over is regarded, therefore, as replacing relational coiling.

At variance with the postulates on which this hypothesis rests are the numerous observations of multiple chromonemata at all stages of mitosis and meiosis, the occasional observation that changes in direction other than those resulting from crossing-over occur in chromosome arms, the finding that the major and minor spirals may coil in opposite directions (38, 92a). It is evident, therefore, that the various and often antithetic conclusions concerning chromosome mechanics are conditioned by the diverse cytological observations on which they rest. Accordingly they must be regarded as provisional until a greater number of data, observational and experimental, are offered to test their validity. In the meantime they serve as important steps in the growing efforts to interpret chromosome structure in terms of behavior.

#### CONCLUSIONS

The observational and experimental evidence reviewed here indicates that the chromonema is a fundamental and permanent component of the chromosome. As a structure persistent through the mitotic cycle, it provides a mechanism for the maintenance of the linear order of the genes, its chromomeric organization furnishing morphological evidence of its linear differentiation. Other theories of chromosome structure, which regard the chromonema as transitory in the mitotic cycle, are inadequate in light of present knowledge of chromosome behavior and genetical function.

With respect to the number of chromonemata per chromosome, the bulk of evidence favors the interpretation that the somatic metaphase chromosome is 4-partite, and that the bivalent of the first

meiotic metaphase is 8-partite. The chromonemata of such somatic chromosomes are coiled in minor spirals, those of the meiotic bivalent in both major and minor spirals. Relatively little is known of the behavior and rôle of the achromatic matrix material with which the contracted chromonemata usually are associated.

Studies of chromosome structure during meiosis have been made primarily on the metaphase bivalents, when the chromonemata are defined most clearly. An accurate knowledge of the organization of prophase chromosomes is essential, however, to critical analysis of such phenomena as synapsis, chiasma formation and crossing-over. In several of the recent studies, efforts have been made to trace the chromonemata through the first meiotic division, but evidence on the structure of leptotene and pre-leptotene chromosomes remains meagre. Additional cytological investigations of these stages will furnish a more substantial basis than now exists for such speculations concerning chromosome mechanics as are indicated in the preceding section of this paper.

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## GLOSSARY ADAPTED IN PART FROM DARLINGTON'S

## "RECENT ADVANCES IN CYTOLOGY"

- anaphase: the stage of a nuclear division subsequent to metaphase and previous to telophase, during which daughter-chromosomes move apart.
- bivalent: a group of two homologous chromosomes.
- chiasma (ta): an exchange of partners amongst four chromatids associated in pairs.
- chromatid: a half of a longitudinally split chromosome.
- diakinesis: the last stage in the prophase of the first meiotic division, immediately before the disappearance of the nuclear membrane.
- diplotene: the stage following pachytene in the prophase of the first meiotic division, during which the chromatids of the tetrad widen out in pairs, so that the four chromatids become plainly visible.
- dyad: the univalent chromosome, composed of two chromatids, at meiosis.
- heterozygote: an organism derived from the union of gametes dissimilar in respect of their chromosomes.
- homologue: one of two homologous chromosomes, i.e., those contributed by the two parents and influencing the same characters.
- interkinesis: the resting stage which may occur between the first and second meiotic divisions.
- interphase: the period between two rapidly succeeding mitoses.
- karyolymph: the nuclear sap, or ground-substance of the nucleus.
- leptotene: the long slender chromosome threads of the early prophase of the first meiotic division, just before synaptic association; also the stage itself.
- meiosis: a form of division involving two nuclear divisions, during which the chromosome-number is reduced from diploid ( $2n$ ) to haploid ( $n$ ).
- metaphase: the stage of division in which the chromosomes lie in a plane at right angles to the axis of the spindle and half-way between the poles.
- nucleolus: a body in the nucleus which disappears during nuclear division.
- moniliform: jointed or constricted at intervals.
- pachytene: the thick bivalent thread (and the stage at which it occurs) produced by pairing of chromosomes in the prophase of the first meiotic division. This stage is followed by diplotene.
- parasynapsis: side-by-side association of chromosomes during nuclear division.
- spindle-attachment: the point in a chromatid which moves first to the pole at anaphase.
- satellite: a segment of a chromosome, separated from the rest by one long constriction if terminal or two if intercalary.
- telophase: the last stage of nuclear division, after movement of the chromosomes has ceased.
- telosynapsis: the alleged end-to-end union of the chromosomes (synaptic mates) in synapsis. The end-to-end arrangement of chromosomes observed in some organisms at late prophase or at metaphase is now regarded as derived from earlier parasynaptic association. See discussion in Cleland's article, *Bot. Rev.* 2, p. 341.

## PHAEOPHYCEAN LIFE-HISTORIES IN RELATION TO CLASSIFICATION<sup>1</sup>

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It is safe to say that our knowledge of no other great plant group has broadened more rapidly and with more startling changes in the present century than has that of the algae. As these plants are of great economic importance, for the most part either indirectly or in the mass, it is not surprising that the bulk of modern researches on algae has either been ecological or plankton studies or has been directed toward problems bearing on classification. Classification is, of course, a common approach to the study of a plant group. In spite of a quite extensive recognition of species differences, marine algae were classified into *Conferva*, *Ulva* and *Fucus* till long after Linnaeus' time and in works as consequential as Sowerby's "English Botany" (First Edit.) and Dawson Turner's "Historia Fucorum" early in the 19th century. Immediately thereafter these groups of species were broken down into genera of the modern type; much later, reassembly of these into tolerably rational families and orders was accomplished by the efforts of several investigators so that orderly compendia became possible, like J. Agardh's "Species, genera et ordines algarum." The classification of Phaeophyceae was the slowest to respond to studies relative to life-history and cytological state. However, about twenty years ago the publications of Sauvageau (19, 20, 21) and but little later of Kylin (10) began to throw such surprising light on life-histories of the kelps that the fundamental classification in this group had to be overhauled. Research in the Phaeophyceae during the last twenty years has concentrated largely on experimental studies. Some purely chemical, some physiological, most of them have been developmental and their result has been so to broaden and redirect our knowledge of the morphology of these plants as to fundamentally alter our major systematic concepts in the group. There has been relatively little strictly taxonomic activity, little of mono-

<sup>1</sup> Papers from the Herbarium and the Department of Botany, University of Michigan, No. 589.

graphic revision, and that which is cytological in trend has largely been crude and applied to the problems of development. Genetical interest has not yet been awakened in the group.

The importance of life-history studies in outlining major features of classification of the Phaeophyceae developed much more rapidly after the discovery of the gametophytes in the kelps. An alternation of similar gametophyte and sporophyte phases had been recognized for *Dictyota* (5) and of dissimilar phases in *Cutleria*, but this did not stimulate any fundamental revision. When Sauvageau, and then Kylin and others, discovered that *Laminaria* and its relatives (kelps) had microscopic oogamous gametophytes, it became clear that an experimental study was needed of every plant lacking, in the morphological sense, either sporangia or gametangia, or at least in each family and genus of a number of species sufficient to fix the character of that group. Latent enthusiasm for experimental studies on marine algae broke out in a large number of papers, first on the kelps and then on other Phaeophycean groups. For various reasons most of these were incomplete studies, since contaminations (chiefly diatomaceous) and maintenance of a suitable rather low temperature rendered it hard to maintain cultures long enough (several months) to get the full history. Cytological confirmation of the significance of the developmental sequences in particular is usually lacking. In fact, the cytological data respecting Phaeophycean life-histories are, from the standpoint of the cytologist, badly worked out, showing every evidence of lack of technical mastery. The material appears to be somewhat difficult to handle, so that at best all that can now be demonstrated is the chromosome count in the subject.

The general result of these cultural studies has been to show that many Phaeophyceae possess phases which are relatively minute in stature. Some of these are cytologically obligatory alternates in the developmental cycle; more generally they are not. In the latter case they may represent ecologically advantageous conditions somewhat comparable to the protonema of a moss. Some have no reproductive functions and only grow to the adult plant directly and vegetatively, although when branching they multiply the points of origin of the more massive growth stage. Others have reproductive functions, and it is not surprising that such individuals should be

repetitional, with the possibility that several generations might succeed each other before the incidence of the massive stage.

Lacking comprehensive evidence to the contrary, it appears that these repetitional phases do not involve any change in chromosomal number. Passing into the massive stage without any such change, they are not to be considered as essential factors in the gametophyte-sporophyte cycle, since the massive stage is either continuous in growth with the minute stage or else bears the same type of reproductive organ.

It is frequently reported that the minute stage is functionally different in its reproduction from the succeeding massive stage growing from it. For instance, where both have plurilocular organs, those on the minute stage function as fertile gametangia while those on the massive stage produce neutral zoöspores. This may well be true as to their functioning under experimental conditions, but that it is always equally true under natural conditions is a dangerous assumption. There is so little known as to the best conditions for maintaining these cultures, particularly for sexual fusions, so little uniformity and reproducibility of results, so much relation perhaps with the season at which they were set up, that inferences should be drawn from them only with the greatest caution. Particularly is it unwise to attribute obligate limitation of sexual functions to such stages when the massive phase bears morphologically similar reproductive organs. The evidence that under other conditions these massive stages may not be sexually fertile is negative in nature and so, on such a question, inconclusive.

It is perhaps well to consider here to what degree the conceptions of sporophyte (a  $2n$  or diploid plant) and gametophyte (an  $n$  or haploid plant) can be applied to the Phaeophyceae. We have no conditions comparable to those in the higher Rhodophyceae where the haploid sexual plant supports an epi- or endophytic diploid multiplicative carposporophyte which is largely parasitic and largely, if not wholly, lacks vegetative tissues. The ameiotic spores of this sporophyte give rise to a second, now independent and diploid tetrasporophyte, similar in vegetative aspect to the gametophyte and climaxing in the meiotic production of spores in tetrads (quartets). Even with this distinctive morphological alternation of three generations the greatest care must be exercised in assumptions involving the haploidy or diploidy of

any tissue. We have genera (like *Callithamnion* and *Spermothamnion*) where sexual organs and tetraspores appear on the same plants; others where the carposporophyte (some *Liagora* and *Phyllophora* species) is closed by the production of tetraspores (presumably meiotic); and finally others (in Bangioideae and the lower Florideae) where meiosis precedes carposporophyte development so that it is haploid from the start and the tetrasporophyte is absent. It is plain from the conditions in the Rhodophyceae that the morphology of the plant is not absolutely linked with the single or double chromosome grouping, wide-spread as such association is among algae and other plants.

In the Phaeophyceae this is equally true. In the Ectocarpaceae, for example, gametangial structures of the purilocular type and sporangial structures of the unilocular type are not infrequently simultaneously found on the same plant. The development of sporangia and gametangia is often successional, either seasonal (*Leathesia*) or morphological as in *Myriotrichia*, where sporangia are borne on the creeping parts and gametangia on the erect axes.

However, such cases do not change the general rule that successive cell divisions giving plurilocular reproductive organs are associated with gamogenesis and the plants bearing them are to be considered morphologically as gametophytes, while simultaneous cytokinesis, segregating nuclei from a multinucleate protoplast, is associated with zoospore production and the plants are morphologically sporophytes. In descriptive morphology and taxonomy they must be so treated or confusion results. The actual physiological behavior of the products of plurilocular and unilocular organs, in relation to sexual or asexual functions and to the production of haploid or diploid individuals, should be kept as a separate problem and not be allowed to confuse the morphological terminology on which the taxonomic arrangement rests.

Parthenogenesis of haploid gametes is reported and when diploid plants produce what morphologically are gametangia, the diploid swimmers therefrom are sexually inert but may reproduce the diploid phase. They are not zoospores in the sense of the haploid swimmers from unilocular sporangia; they more nearly correspond to the diploid zoospores produced if meiosis is omitted from sporangial development. On the other hand, sexual functions have

been ascribed to the products of sporangia, but such reports are not so well substantiated except in that old climax group, the Fucaceae. As differences between sporophyte and gametophyte become more marked, the association of form with type of reproductive body and chromosome number becomes more rigid. The shift to the condition seen in the Fucaceae is abrupt, and the group isolated, for we lack stages in the final suppression of a filamentous gametophyte and we lack plants showing a comparable heterospory. It seems that the protoplasmic divisions in the reproductive organs are of the simultaneous rather than the successive type (except for a vestigial wall formation late in antherozooid production), and yet it seems best to consider them as gametangial. We probably can not argue from such a case the plausibility of reports of sexual fusions between products of sporangia (as in Ectocarpaceae) and these reports will have to stand on such merit as confirmatory studies disclose.

#### TAXONOMIC REVISION

It long ago became clear that the whole systematic structure of the group needed reconsideration, and the writer made some suggestions toward this end in 1920 (32). However, his outline was not sufficiently complete to serve all practical needs and with later research became obsolete. Setchell and Gardner (31) and Kylin (12, 13) in turn offered their preferences, the former essentially from the systematic, the latter from the developmental standpoint. Kylin's more modern version seems to offer a sufficiently broad foundation to serve for a systematic reorganization of the Phaeophyceae. The first necessary change is cancellation of the primary segregation into Phaeosporales and Cyclosporaes as natural orders. Certainly there are additional groups worthy of ordinal rank, and surely the group Cyclosporaes, founded for the Fucaceae, would be artificial if made to include the other oogamous families.

We are offered three classes: Isogeneratae, Heterogeneratae, Cyclosporeae, which last is restricted to the Fucaceae and apparently in an ancient and isolated group.

*Isogeneratae*.—The Isogeneratae, with sporophyte and gametophyte generations alike in form, and the Heterogeneratae, with the generations different, are not at all clearly characterized. This separation is useful if interpreted as designating tendencies rather



than a completed segregation. Otherwise the terms are misleading. In the Isogeneratae five orders are included. The Ectocarpales includes filamentous or somewhat crustose types with (at least in part) intercalary divisions of the filaments, these almost exclusively transverse. To us *Ectocarpus* is the most familiar genus (7, 8, 17, 29). The Sphacelariales are mostly filamentous, but these filaments grow from prominent apical cells and the segments frequently divide lengthwise in a regular polysiphonous manner (4, 16). Our common genera are *Sphacelaria* and *Cladostephus*. The Cutleriales have trichothallic growth and develop a parenchymatous blade or a disc-like thallus. While *Zanardinia* conforms, *Cutleria* has the sporophyte so much smaller than the gametophyte as to make this order an anomaly in a group called Isogeneratae (12, 27). American representatives are lacking. The Tilopteridales appear morphologically to belong in the Isogeneratae; the plants are filamentous with intercalary growth, but the intercalary gametangia and sporangia show such peculiarities that the degree of sexual evolution is quite uncertain (25). The Dictyotales, which appear to be a climax group, grow like the Sphacelariales from an apical cell or cell row but almost exclusively exhibit a flat blade; they are oogamous. In the sporangia no divisions occur after meiosis and the spores are not motile (5). In the warmer American waters *Dictyota* and other genera are found.

*Heterogeneratae*.—The Heterogeneratae are divided into subclasses on the basis of their type of growth. On the one hand stands a group with the thallus built up of one or more filaments and their lateral branches (Haplostichineae), and on the other, one with an axis divided and enlarged by longitudinal walls to a parenchymatous structure (Polystichineae). Both these lines show stages in sexual differentiation from isogamy to oogamy. In the simplest order of the Haplostichineae, the Chordariales, the fully developed thallus is usually of the multiaxial type. It bears sporangia in this state and sometimes plurilocular organs which are morphologically gametangia. Since it appears that the structures developed from the zoöspores may be microscopic filamentous plantlets reproducing by gametangia, it is probable that we have a facultative alternation of dissimilar generations in the order (11, 24). However, since the fully developed plants often bear gametangioïd structures whose infertility is not universally assured,



and since the diminutive phases often produce the fully developed plants vegetatively, it is quite misleading to assume an obligate alternation. The probability is great that the plants persist without either meiosis or zygosis in a diploid state. But for its ponderosity, Sauvageau's noncommittal term of plethysmothalle is more suitable (28). Kylin includes Chordariaceae, Elachisteaceae and Spermatocnaceae in the order, and the genera prominent on our coast include *Chordaria*, *Mesogloia* and *Elachistea* (12, 18, 27).

The Sporochnales are cited as sporangium-bearing in the fully developed plants and as producing plurilocular gametangia on the diminutive phase (22, 23). However, this is known for *Carpomitra* and not for *Sporochnus*, cultures of which have remained sterile. The large plants are morphologically rather specialized, the somewhat club-shaped branchlets bearing tufts of hairs in terminal pits. We find *Sporochnus* in American tropical waters. The climax order of the subclass is the Desmarestiales. In plants of this group there are one or more heavily corticated axial filaments and the branchlets often bear more or less deciduous assimilatory filaments in tufts. Two families are accepted by Kylin: Desmarestiaceae and Arthrocladiaceae (12, 27, 30). Both have microscopic plantlets which in the Desmarestiaceae are definitely said to be oogamous gametophytes but in the Arthrocladiaceae they may have lost their effectiveness. The genera *Desmarestia* and *Arthrocladia* are both found in northern American waters.

The Polystichineae likewise comprise three orders. Throughout, there is a dominant tendency to increase the bulk of the plant by intercalary cell division though filaments do appear in many species in some part of the construction. The Punctariales are reported as having a microscopic phase bearing gametangia; it is to be viewed in much the same light as that of the Chordariaceae, for some plants show gametangioïd structures on the massive phase, some show sporangia on the microscopic phase, and the smaller may pass into the larger vegetatively (12, 26). The gametangia are alike and zygosis is isogamous; Asperococcaceae, Myriotrichaceae and Striariaceae probably belong in the order, and all families are represented by the typical genera in our northern Atlantic flora.

The Dictyosiphonales are represented by the Dictyosiphonaceae, the only family. The large plants grow from apical cells; segmentation is followed by intercalary divisions. These plants bear

sporangia; the zoöspores develop a filamentous phase which normally appears to be a gametophyte bearing isogametes in small plurilocular gametangia. The alternation ordinarily appears to be an obligate one (12, 26).

The climax of the subclass is the Laminariales (Laminariaceae the only family) where growth is intercalary throughout and extensively parenchymatous, though rudimentary filamentous tissues do persist. Obligate alternation of a microscopic oogamous gametophyte and a very large sporophyte seems general. This is by far the most extensively studied family in the higher algae with respect to life-histories (1, 2, 3, 6, 10, 11, 12, 14, 15, 19, 20, 21). It is a northern family, with *Laminaria*, *Chorda* and other genera on the east coast.

*Cyclosporeae*.—The final class, Cyclosporeae, also comprises a single order Fucales and family Fucaceae. Growth is initiated by an apical cell group which develops a parenchymatous tissue, often about a filamentous medulla. Fertile areas seem to be progressively restricted toward branch tips or specialized branches, where sporangia are formed in conceptacular pits associated with paraphyses. In the northern flora *Fucus* is common.

The sporangia are dimorphic in arrangement and in size, the larger with one nuclear division after meiosis, the smaller with three. In some cases not all of the megasporangium-derived nuclei persist. The cytokinesis after division segregates 8 cells in the larger sporangia and 32–64 in the smaller. These are discharged, enveloped in the sporangial walls which soften in the water to liberate large motionless cells and small active flagellate swimmers which function as eggs and antherozoids, respectively, the zygote giving rise to the diploid sporophyte. It is obvious that this plant, lacking any independent haploid phase, has an extremely reduced gametophyte. Phanerogams offer an analogous condition.

It seems possible that the evolution of a minute protonemal phase vegetatively continuous which the adult massive gametophyte has been followed by partial and later complete shifting of reproductive fertility from the larger to the smaller stage and finally even the elimination of the large gametophyte. We have no haploid phase in the large form which has sex differentiation evolved to the degree of oogamy, except in the Dictyotales, but this

has apparently occurred independently in all of the lines with reduced gametophytes.

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#### GLOSSARY

carposporophyte: a structure among the red algae which arises from fertilization of an egg in a carpogonium by a spermatium and which bears carpospores. The latter produce new plants which in some forms bear sex cells again. In most cases, however, they give rise to plants known as tetrasporophytes which produce asexual tetraspores and these then develop plants which bear sex cells.

cytokinesis: division of the extra-nuclear portion of the protoplasm.

gametangia: differentiated cells or cell groups in algal filaments which produce gametes or sex cells.

gamogenesis: formation of sexual cells.

heterogamy: the state of male and female sex cells being distinguishable as contrasted with isogamy wherein they are alike and usually motile.

isogamy: see heterogamy.

oögam: the state of differentiation between the sexual cells where the female cells alone completely lack organs of motility.

Phaeophyceae: the brown algae, one of the great groups of algae, most conspicuous in cold temperate waters.

plurilocular: many-celled, with reference to sex organs.

protonema: the thread-like growth issuing from the spores of mosses and upon which the conspicuous plants are developed as lateral or terminal shoots.

Rhodophyceae: the red algae, one of the great groups of algae, inhabiting primarily temperate and warm waters.

sporangium: a unicellular structure in the algae within which a spore, or by free cell division several spores, are produced.

trichothallic: a type of growth characterized by a fringe of hairs along the growing margin or a tuft terminating the branch. Growth proceeds from the meristematic activity of the cells at the bases of these hairs.

zygosis: fusion of sex cells.

† A complete citation of literature on the relation of structure and life history to the classification of the Phaeophyceae, even if limited to recent papers and the most essential older works, would include 200-300 items. Those cited are merely examples fairly representative of the plants concerned and of the countries in which most of the work has been done. Through the bibliographies which they contain (especially Kylin 1933) most of the important papers can be located.



# THE BOTANICAL REVIEW

VOL. II

DECEMBER, 1936

No. 12

## CONTEMPORARY UNDERSTANDING OF EMBRYO-SAC DEVELOPMENT AMONG ANGIOSPERMS

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According to a generally wide-spread and undoubtedly well-founded conception, the female gametophyte or embryo-sac among angiosperms is to be regarded as a structure which has passed through considerable evolutionary change and is now reduced to a few cells. It is considered as originally having been an independent generation, which among angiosperms has become an organ of the sporophyte.

It is generally observed that structures which have thus experienced considerable evolutionary change and reduction show also a high degree of variability. The embryo-sac of angiosperms is no exception and it is the purpose of the following discussion to set forth clearly the variability which occurs in its development and structure and to derive therefrom some general conclusions.

Nothing new is presented to the literature upon the subject by this paper. Attention may be directed here only to the system of embryo-sac types proposed by Palm (53) and Chiarugi (12) and to contributions published by various other authors. A new treatment may, however, be appropriate if it is concerned not only with a correlation of the old and most recent discoveries, but if it is devoted primarily to a critical distinction between well established conceptions and those that are doubtful.

It is fitting, toward this end, if we describe first those types of embryo-sac development in angiosperms which may be regarded as well established. In another part we may consider those cases that are doubtful and which merit further investigation. Certain claims may also be discussed there which heretofore have been regarded as thoroughly to be depended upon or on which particular doubt has not been cast.

This article was translated by the editors from the original German.

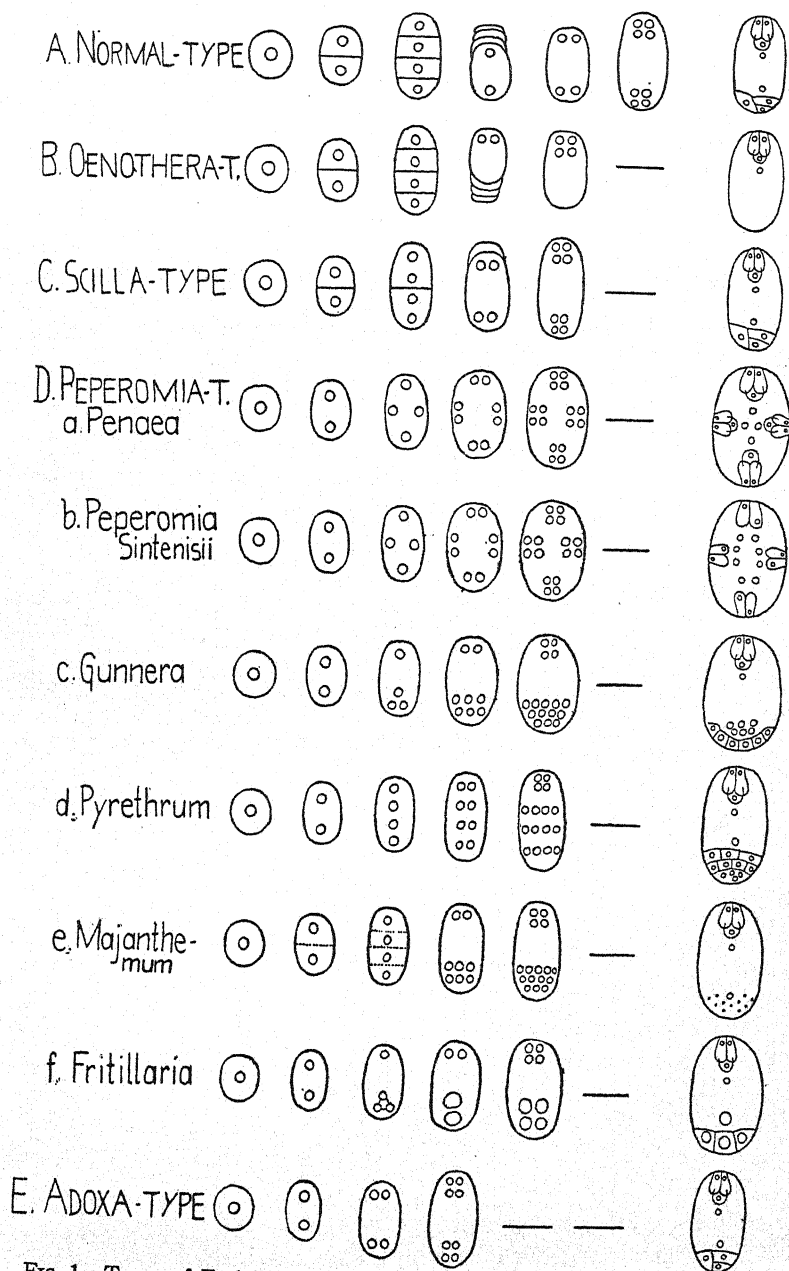


FIG. 1. Types of Embryo-sac Development among Angiosperms.

## THE WELL-ESTABLISHED TYPES OF EMBRYO-SAC

The limits and characteristics of developmental types discussed under this heading are based upon Palm's account (53). This author employs the number of successive nuclear divisions involved in the course of embryo-sac development from the embryo-sac mother-cell to formation of the egg-cell, as the principal basis for his classification. As a secondary criterion, he uses the number of macrospores involved in formation of the embryo-sac.<sup>1</sup>

Attention may be called at this point to the attempt of Rutgers (59) to express by a detailed system the great variation in embryo-sac development and to represent by certain formulae the types which he recognized. This attempt must be regarded as scarcely successful, primarily because the author attributes to the macrospore a new and entirely inappropriate significance. A similar attempt of Radermacher (58) can also be looked upon as unsuccessful. Chiarugi's system (12), on the contrary, contains important ideas which will be of value in the following discussion.

## THE NORMAL-TYPE

The normal-type is characterized, first by the fact that the embryo-sac mother-cell undergoes 5 divisions before formation of the egg-cell, and secondly by the fact that the embryo-sac originates from only 1 macrospore.<sup>2</sup> Two phases can be distinguished in this course of development (Fig. 1, A). The first leads to formation of the macrospore and consists of 2 successive divisions which involve conversion of the diploidy of the macrospore mother-cell into the haploidy of the macrospores. This phase may be briefly designated in the following discussion as spore-formation (sporogenesis of Chiarugi (12)). In the second phase, which usually is carried out by only 1 macrospore, the foundation for the fully developed and fertilizable embryo-sac is laid down by 3 successive nuclear divisions. This stage of development, the embryo-sac for-

<sup>1</sup> All methods of embryo-sac development described for those plants that have suffered loss of normal sexual reproduction (apomictic plants) are omitted in the following discussion. In addition to Schnarf's summary (61, 460-469), see, in particular, Gustafson (29).

<sup>2</sup> I am employing the terms macrospore and macrosporangium rather than the newer and more frequently used megaspore and megasporangium because the former are older. Philological considerations do not permit rejection of old long-established terms and their substitution by new ones.



mation,<sup>3</sup> is characterized by considerable growth and by formation of the micropylar and the chalazal poles. Directly after the first division of the macrospore nucleus the young embryo-sac elongates considerably and a large vacuole always forms between the 2 daughter nuclei, that is, between the primary micropylar nucleus and the primary chalazal nucleus. This vacuole enlarges during the 2 subsequent nuclear divisions, after whose completion there are 4 nuclei at each pole. A complex then develops from each of these quartettes, or oangia as they are called by Chiarugi, consisting of 3 cells and 1 free nucleus. The one arising from the chalazal quartette comprises 3 antipodal cells and the lower polar nucleus while the complex at the micropylar end consists of the egg-cell, the 2 synergid cells and the upper polar nucleus. We can regard it as probable that the synergids on the one hand, and the egg-cell and the upper polar nucleus on the other hand, represent sister-nuclei; at least this is definitely shown in certain cases while no reliable observations are at hand to substantiate the idea of any other origin.

Within the normal-type we can find different variations which are exhibited in part by sporogenesis and selection of the functioning microspore and partly in embryo-sac formation.

In the first group of variations there belongs, for example, the degeneration, after the first (heterotypic) division and before the second (homotypic) division, of one of the 2 daughter-cells, the one nearer the chalazal end. In this manner a row of 3 cells is formed, only two of which, however, merit the name macrospore. It is obviously incorrect when Rutgers (59), in his description of embryo-sac development faulty also in other respects, attributes great significance to this modification in the selection of the functioning macrospore.

The normal-type shows greater variation in the second phase, that of embryo-sac formation. We find, in particular, that its antipodal half rather frequently appears altered by degeneration or advancement. The former is expressed by a slight reduction of divisions which the primary chalazal nucleus undergoes and the latter by an increase in the antipodals. It is of interest that in

<sup>3</sup> Within formation of the embryo-sac, Chiarugi (12) distinguished 2 stages, namely, somatogenesis, characterized by formation of the vacuole and by the first mitosis of the macrospore nucleus, and gametogenesis, during which the last 2 divisions take place.

various cases involving a greater number of antipodals in the fertilizable embryo-sacs, three of them have been shown to be of only temporary existence during the course of development.

#### THE OENOTHERA-TYPE

This type, designated as the *Codiaeum*-type by Palm (53),<sup>4</sup> is characterized, first by the fact that the embryo-sac mother-cell undergoes 4 divisions up to formation of the egg-cell, and secondly by development of the embryo-sac from a single macrospore. This type, confined according to all indications to the Oenotheraceae, resembles the normal-type in sporogenesis; in contrast thereto, however, and with few exceptions, the micropylar nucleus is the one which always develops into the embryo-sac (Fig. 1, B). This anomalous selection of the functioning macrospore, largely fixed by heredity, is apparently associated with the complete suppression of the chalazal half of the normal embryo-sac. The functioning macrospore first undergoes considerable growth, resulting in the displacement of the macrospore-nucleus by a vacuole toward the micropylar pole of the young embryo-sac. At that point occur the 2 divisions giving rise to the normal quartette which in the fully developed embryo-sac consists of the 2 synergids, the egg-cell and 1 polar nucleus.

#### THE SCILLA-TYPE

In contrast with the *Oenothera*-type, which is known in only a small number of forms, the *Scilla*-type (Fig. 1, C) has apparently developed independently from the normal type in many different classificatory groups. Its development is accomplished by 4 successive nuclear divisions from the embryo-sac mother-cell up to formation of the egg-cell. Compared with the *Oenothera*-type, however, 2 macrospores are involved in the formation of the embryo-sac. Attention may be drawn, in particular, to the fact that after the first (heterotypic) division a cross-wall is formed, but that wall formation ceases or is only transitory after the second (homotypic) division. The 4 macrospore nuclei are situated,

<sup>4</sup>I prefer (61, 189) to reject this name and to use in its place the expression *Oenothera*-type, primarily because of the doubtfulness of Arnold's data concerning *Codiaeum*. Lundberg (47) has shown that *Codiaeum variegatum* follows the normal type.

therefore, in 2 adjacent cells, the micropylar one of which usually degenerates.

While in the normal-type and *Oenothera*-type sporogenesis and embryo-sac formation are clearly distinct processes, in the *Scilla*-type they merge into one another indistinguishably. The second division is the last division in sporogenesis and the first one (somatogenesis according to Chiarugi (12)) of embryo-sac formation.

As in the normal-type, there are variations within the *Scilla*-type also. We may mention here only those to which attention has been called by Went's studies upon numerous members of the Podostemonaceae (72-75). Of the daughter-cells formed by the first maturation (heterotypic) division, the micropylar cell soon degenerates but its former existence is long indicated by a kind of cap over the embryo-sac. The embryo-sac is formed in the lower daughter-cell by the 2 macrospores which have arisen from the second maturation division. The 2 macrospore nuclei move toward the poles of the young embryo-sac. By 2 successive divisions the micropylar nucleus produces the 4 nuclei which form the egg apparatus and the upper polar nucleus. The primary chalazal nucleus soon degenerates, on the contrary, and formation of a quartette in the chalazal region is omitted. It is particularly noteworthy that in the Podostemonaceae the completed embryo-sac is scarcely any larger than the embryo-sac mother-cell.

Additional examples of the *Scilla*-type of embryo-sac, modified by restricted development of the antipodal region, are furnished by members of the Alismataceae investigated by Dahlgren (17), viz: *Alisma Plantago*, *Alisma natans*, *Echinodorus ranunculoides* and *Damasonium alisma*. In these cases embryo-sacs with 5 or 6 nuclei develop as a result of the fact that the primary chalazal nucleus of the embryo-sac divides only once or not at all.

#### THE PEPEROMIA-TYPE

The *Peperomia*-type is characterized, first by participation of 4 macrospore nuclei in its development, and secondly by the fact that up to formation of the egg-cell the nucleus of the embryo-sac mother-cell undergoes 4 successive divisions. This type, furthermore, is composite in so far as it includes a variety of modifications. The most important of these variations which may be recognized within the type are distinguished here as its forms and

the positions of the 4 macrospores within the embryo-sac mother-cell play a significant rôle in their recognition.

1. *Penaea-Form*.—This form, observed in the Penaeaceae and certain species of *Euphorbia*, exhibits the following course of development (Fig. 1, D. a.). No walls are formed between the macrospore nuclei resulting from the 2 meiotic divisions. When this 4-nucleate cell enlarges and a central vacuole forms within it, the 4 macrospore nuclei are so distributed that one lies near the micropylar pole, another near the chalazal pole and two are laterally located opposite one another. Each one of the 4 nuclei then produces a quartette by means of 2 divisions and in the completed embryo-sac each of these quartettes consists of 3 cells and 1 free nucleus. There are, then, in addition to the egg apparatus, 4 polar nuclei and 3 entirely similar groups each of which consists of 3 cells.

2. *Peperomia-Form*. Here, too (Fig. 1, D. b.), 4 macrospore nuclei are formed in the first 2 divisions and walls develop which soon disappear, or none are formed at all. The 4 nuclei are distributed approximately as the apices of a tetrahedron and, along with simultaneous growth of the embryo-sac, they undergo 2 divisions so that altogether 16 nuclei develop which are at first free. Further development of the embryo-sac is different among various species of *Peperomia*. Formation of 2 cells, the egg-cell and the synergid, can generally be definitely established at the micropylar pole. The other 14 nuclei, on the contrary, undergo a variety of further developments. They may all remain free (*Peperomia hispidula*) or peripheral cells may be formed. In *Peperomia Sintenisii* 8 such cells develop, in other species fewer, and the number of free nuclei in these cases is correspondingly smaller.

3. *Gunnera-Form*. The form (Fig. 1, D. c.) which is characterized by species of *Gunnera* develops a 16-nucleate embryo-sac by means of 4 synchronous nuclear divisions. By means of 2 successive divisions a quartette of nuclei arises at the micropylar pole from one of the 4 macrospores, which quartette produces the egg apparatus and 1 free nucleus. Six of the other 12 nuclei give rise to 6 antipodal cells at the chalazal end, and the remaining 6 free nuclei behave as polar nuclei.

4. *Pyrethrum-Form*. Palm found the following course of development in *Pyrethrum parthenifolium*. A binucleate cell de-

velops as the result of the first meiotic division and a 4-nucleate cell from the second division (Fig. 1, D. d.). This cell elongates and the 4 macrospore nuclei lying in tandem become separated from one another by vacuoles. Each of them then undergoes 2 more divisions. From the micropylar quartette there arise the egg apparatus and the upper polar nucleus. The 12 other nuclei form the lower polar nucleus and a large antipodal apparatus consisting of one 4-nucleate cell and 7 uninucleate cells.

5. *Majanthemum-Form.* In *Majanthemum bifolium* Stenar (68) found the following course of development (Fig. 1, D. e.). Four macrospore cells first arise as a result of the 2 maturation divisions. The walls separating these cells become absorbed, however, and all 4 macrospore nuclei take part in forming the completed embryo-sac. Eight nuclei arise after the following division, two of which we find in the micropylar region, six of them in the chalazal region and a large vacuole between the two groups. After the next division the micropylar nuclei form the egg-apparatus and the upper polar nucleus. So far as the early degeneration of the antipodal region permits of determination, one of the 12 chalazal nuclei apparently becomes the lower polar nucleus and the remaining 11 form antipodal cells.

6. *Fritillaria-Form.* The description of this form (Fig. 1, D. f.) is based upon observations of Bambicioni (2) with respect to *Fritillaria persica*. In this plant the embryo-sac mother-cell becomes 4-nucleate as a result of 2 meiotic divisions without the formation of vacuoles. The 4 macrospore nuclei are then distributed approximately as has already been described for *Penaea*, but one of them soon appears at the micropylar end and the other three at the chalazal pole. All 4 nuclei then undergo division simultaneously but the 3 chalazal nuclei exhibit the special feature of fusion of their spindles. This third division results, then, in 2 haploid nuclei at the micropylar pole and 2 triploid nuclei at the chalazal pole. These 2 pairs of nuclei then become separated from one another by a large vacuole and the fourth nuclear division results in a haploid quartette (egg-apparatus and upper polar nucleus) and a lower triploid quartette (antipodals and lower polar nucleus). Since the entire development from embryo-sac mother-cell to egg-cell is consummated in 4 successive divisions and since all 4 macrospores participate in formation of the embryo-sac, I am not in-

clined to include this form in the general conception of the *Peperomia*-type. The especially remarkable and interesting feature of the *Fritillaria*-form lies in the fact that in the transition from the *Peperomia*-type an embryo-sac is produced apparently from the usual 8 nuclei.

This course of development appears to be established for a number of members of the Lilioideae (in the sense of K. Krause in *Naturl. Pflanzenfam.* 2. Aufl.) other than *Fritillaria persica*, e.g., *Tulipa praecox* (3), *T. Gesneriana* (4) and species of *Lilium* (3, 14). As is indicated by certain features in the older literature, the same course of development probably applies also to other members of the Lilioideae. *Erythronium dens canis* also shows the characteristic distribution of the macrospore nuclei (1 and 3) and seems to follow the *Fritillaria*-form (31). The *Fritillaria*-form is apparently generally characteristic of the Lilioideae (in K. Krause's conception of the group). This fact is not altered by the occurrence of certain exceptions as, for example, in *Tulipa Gesneriana*. In this case the distribution of nuclei previous to the third division is sometimes different, not 1 and 3, but 1 and 1 and 2, i.e., with a macrospore nucleus in the micropylar region, another approximately in the center, and two at the chalazal end; in the third division only the 2 chalazal spindles fuse, and the resulting chalazal nuclei are diploid rather than triploid. In the case of a cultivated plant, such as *Tulipa Gesneriana*, we cannot attribute particular significance to such an anomalous course of development. In the case of *Tulipa silvestris* (3) it is more difficult to pass judgment. Here the 4 macrospore nuclei gather at the micropylar pole and form, after the next division, a group of 7 cells and 1 polar nucleus.

The *Fritillaria*-form is found also in *Euphorbia dulcis*<sup>5</sup> (8) as well as among the Lilioideae. It is very probable, furthermore, that certain courses of development which are attributed in the of the *Peperomia*-type, e.g., *Myricaria germanica* and species of literature to the *Lilium*-type, in reality follow the *Fritillaria*-form *Piper* (62, 93, 94).

#### THE ADOXA-TYPE

The *Adoxa*-type (Fig. 1, E) is to be characterized, as is the *Lilium*-type in the old sense, first by 3 successive nuclear divisions

<sup>5</sup> Bambicioni (2) names the development which she found in *Fritillaria* as the *Euphorbia dulcis*-type, a designation, not recognized here, which fails to account for important details of Carano's description.

from the embryo-sac mother-cell to the egg-cell, and secondly by participation of all 4 macrospore nuclei in formation of the female gametophyte. Under the heading of *Adoxa*-type, first so termed by Bambicioni, we must include all those courses of development which formerly were included in the *Lilium*-type, but only so far as they have not been shown by recent investigation to belong to the *Fritillaria*-form of the *Peperomia*-type. That there actually are forms which conform to the *Adoxa*-type, we cannot deny in view of our present understanding. *Adoxa Moschatellina* certainly belongs to this type (40, 46).

We may well refer here to a recently described modification of the *Adoxa*-type. In *Gagea lutea*, according to Stenar (67), 4 macrospore nuclei are formed in a row by the 2 maturation divisions in the embryo-sac mother-cell. Upon growth of the embryo-sac, 2 of the nuclei are displaced toward the micropylar pole and 2 toward the chalazal end. (Only occasionally is there a 1 and 3 distribution.) The third division results in 8 nuclei and these form the embryo-sac; or, as more frequently seems to be the case, the lowest macrospore nucleus degenerates and does not undergo the last division, so that only 2 antipodals appear.

## THE DOUBTFUL TYPES

### THE LILIUM-TYPE

Older authors and some of the more recent ones indicate the following type of development as occurring in numerous species, e.g., of *Lilium*, *Fritillaria*, *Tulipa*, *Adoxa*, *Piper* and of other genera. Four macrospore nuclei are formed by the 2 meiotic divisions without formation of walls; by the next division these nuclei produce an 8-nucleate embryo-sac. The *Lilium*-type is characterized, then, by 3 successive divisions and by the fact that all 4 macrospores participate in the development as well as by the fact that the two developmental phases, sporogenesis and embryo-sac formation, so sharply distinguished in the normal type, are even more completely merged than in the *Scilla*-type.

We can understand a critical attitude toward the *Lilium*-type in the sense as here given from the fact that such genera as *Lilium* and *Fritillaria*, which previous to Bambicioni's and Cooper's inves-

tigations had often been studied and were regarded therefore as unquestionable examples of the *Lilium*-type, now appear to be representatives of a modified *Peperomia*-type. Even older investigations showed certain details which did not conform entirely to the accepted development of the *Lilium*-type, such as the remarkable distribution of the 4 macrospore nuclei with one at the micropylar pole and 3 at the chalazal end. Furthermore, it was peculiar that the chalazal nuclei should frequently show a greater chromosome number than those at the micropylar end. These noteworthy facts were sometimes neglected by the older authors as anomalies, or accessory hypotheses were introduced to explain them (cf. Schnarf, 1929, pp. 205-207).

Bambicioni herself has called attention to certain findings of Frisendahl (23) in his study of *Myricaria* which indicate that the development in this plant follows that of *Fritillaria* (62, 93, 94). It may also be mentioned that certain angiosperms of entirely different relationship show similar peculiarities, which indicate that they belong not to the *Lilium*-type but to the *Fritillaria*-form of the *Peperomia*-type. As certain stages indicate, even the *Lilium*-type described for *Piper* may prove after more thorough investigation to represent the *Fritillaria*-form. No evidence is afforded us at present by the descriptions of embryo-sac development in *Adoxa moschatellina* (Jönsson, 1879; Lagerberg, 1909) or even in *Armeria* and *Statice* (Dahlgren, 1916), where the development has been recorded as of the *Lilium*-type. It would be very desirable if such claims could also be reinvestigated.<sup>6</sup>

Purely theoretical considerations from the viewpoint of comparative morphology also give us occasion for demanding a re-examination of all claims concerning the occurrence of the *Lilium*-type in its former sense. In nearly all courses of embryo-sac development we observe that the characteristic basic feature of the completed embryo-sac, namely, the quartette of 3 cells and 1 free nucleus, arises in such a fashion that a macrospore divides and its daughter-cells produce the quartette (normal-type), or a macrospore itself becomes the initial nucleus of the quartette (*Oenothera*-type, *Scilla*-type, *Peperomia*-type). Is it only in the old conception of the

<sup>6</sup> Certain researches on species of *Aloë* justify me in regarding Givelli's (25) claims as wholly unreliable when he says that *Aloë arborescens*, *A. Todari* var. *praecox*, *A. caesia*, *A. Varvari* and *A. ciliaris* follow the *Lilium*-type in their development.



*Lilium*-type that 2 macrospores are supposed to take part in forming the quartette, i.e., 2 entities each of which possesses the ability, phylogenetically acquired, of independently evolving a gametophyte generation?

In a paper whose object is a critical presentation of the actual facts, I cannot go so far as to characterize all claims concerning the *Lilium*-type as unfounded, and this in spite of the theoretical considerations only briefly noted here. I have already expressed this conservative viewpoint by referring to and describing the *Adoxa*-type in the first part of this paper.

#### THE CYPRIPEDIUM-TYPE

This type is founded upon the description by Pace (50) of embryo-sac development as it occurs in *Cypripedium spectabile*, *C. parviflorum*, *C. pubescens* and *C. candidum*.<sup>7</sup> According to this description, the embryo-sac mother-cell divides into 2 cells during the first maturation division, whereas after the second division wall formation does not take place. As in the *Scilla*-type, 2 macrospore nuclei are involved in formation of the embryo-sac. A vacuole develops between them in this case also and by the following division 4 nuclei are formed, two of which are at first located at the micropylar pole and two at the chalazal pole. By a rearrangement which then ensues, one of the chalazal nuclei is supposed to move toward the 2 micropylar ones and together with them to form the egg-apparatus, while the second chalazal nucleus serves as a polar nucleus.

This description, though nicely depicted in numerous drawings, nevertheless invokes some criticism from several viewpoints. The claim, for instance, that 1 chalazal embryo-sac nucleus forsakes its position, moves toward the micropylar region and behaves there as a synergid nucleus, must be demonstrated in all its stages in order to be acceptable. Rutgers (59) has referred to this and other weaknesses of the *Cypripedium*-type in a well-founded criticism which is based primarily upon certain figures in Pace's work. Francini's (21) recently published findings respecting *Cypripedium Lecanum* indicate that this criticism is wholly justified. In Francini's species an 8-nucleate embryo-sac is developed according to

<sup>7</sup> *C. spectabile* = *C. reginae* = *C. hirsutum*. The last is used in Gray's Manual, ed. 7.

the *Scilla*-type, or one of fewer nuclei arises by imperfect development of the chalazal half. The mid-European *C. calceolaris* also exhibits a modification of the *Scilla*-type, as is indicated by unpublished discoveries of Ernst Oberhammer.<sup>8</sup> Finally, Prosina (56) found the *Scilla*-type also in *Cypripedium guttatum*.

There are still other cases reported in the literature where it is claimed that by 3 successive divisions a 4-nucleate embryo-sac arises in whose formation 2 macrospores are involved. According to Magnus (48), a 4-nucleate embryo-sac is supposed to develop from 2 macrospore nuclei in *Podostemon subulatus*, *Hydrobium olivaceum* and probably in *Farmeria metzgeroides*. The development in these cases is said to be such that subsequent to division one of the 2 macrospores forms the 2 synergids and the other forms the egg-nucleus and the single polar nucleus. This description appears more likely and the work of Magnus shows no stages which contradict the course of development he claims to have observed. The possibility appears to me, nevertheless, that Magnus overlooked the lower macrospore nucleus and its residuum after degeneration, structures which were found by Went in so many other species of the Podostemonaceae.

#### THE DICRAEA-TYPE

The *Dicraea*-type of Palm (53) is based upon the account of *Dicraea elongata* in Mangus' work on the Podostemonaceae (48). Certain ideas raised also in connection with *Podostemon* and other plants are of interest in this connection. The *Dicraea*-type appears uncertain, however, because Magnus himself admits that insufficient material was at his disposal. Further consideration of the *Dicraea*-type consequently appears out of place.

#### THE PLUMBAGELLA-TYPE

The *Plumbagella*-type, established for *Plumbagella micrantha*, *Plumbago capensis*, *P. pulchella*, *P. zeylanica* and *Ceratostigma plumbaginoides*, is reported to involve 2 successive divisions (15, 16). These, which at the same time are maturation divisions, supposedly give rise to 4 macrospore nuclei. One of the latter becomes the egg-nucleus, another the single antipodal cell and the other two remain free as polar nuclei. This, however, is only the general

<sup>8</sup> I have seen convincing preparations of this myself.

rule. Dahlgren has found a relatively large number of variations and attention may be directed here to the following: 1. No antipodals, 3 free nuclei; 2. The antipodals variously distributed and sometimes converted to a degree into synergids; 3. More than 4 nuclei; 4. Eight-nucleate embryo-sac.

Without question this variability affords a basis for criticism which would have particular significance with respect to the *Plumbagella*-type. This may be regarded in the first place as evidence that the phylogenetic development of higher plants is directed toward suppression of the gametophyte and the ultimate elimination of the antithetic alternation of generations. This interpretation has brought forth the idea that, at least in the female sex of *Plumbagella*, those evolutionary stages have been achieved which the Metazoa exhibit. Because of these facts it is fitting for those who profess these ideas that they carefully examine all arguments. It may be that in the case of *Plumbagella* we have not yet recognized the normal course of development, since we have disregarded certain observed phases as accidental variations and insignificant abnormalities.

These ideas have recently been conspicuously supported by the investigations of Haupt (20) who describes the following course of development for *Plumbago capensis*, a species which has been studied also by Dahlgren. The 4 macrospore nuclei, which arise without wall formation in the embryo-sac mother-cell, are at a certain stage so distributed that one is located at the micropylar end, another at the chalazal end and two are located at the sides. They appear to be pushed toward the wall by vacuole formation. Each of these nuclei divides into two and the resulting pairs have at first similar positions. During the course of further development one of the 2 micropylar nuclei is regularly separated by a thin membrane and the resulting cell constitutes the egg-cell. Four of the remaining 7 nuclei enlarge, move toward the center and fuse, forming the secondary embryo-sac nucleus. The remaining 3 nuclei usually degenerate but sometimes they resemble the egg-nucleus in so far as lenticular cells are formed about them. One, 2 or even 3 cells may be formed in this manner. It may be conjectured that 1 nucleus of each pair in the 8-nucleate stage behaves as a polar nucleus whereas the other becomes the nucleus of an egg-cell. Of the 4 egg-cells only the one nearest the micropyle is

constant and capable of functioning; because of premature degeneration the other three usually do not complete their development.

In any event, Haupt's findings demand a re-examination of the *Plumbagella*-type, especially so because a number of the variations described by Dahlgren fit into the course of development mentioned by Haupt.

#### THE GARCINIA-TYPE

Information concerning embryo-sac development in *Garcinia Kydia* and *G. Treubi* is founded upon Treub's account (70). According to this author, the development in these species follows that of the normal-type as far as the 4-nucleate state, when 2 nuclei are located at the micropylar end and 2 others in the chalazal region. Only one of the micropylar nuclei then divides and is used in the formation of the synergids; the other nuclei are reported to remain undivided, the second micropylar nucleus functioning as the egg nucleus and the two at the chalazal end as polar nuclei. Palm (53) has already called attention to the weaknesses of this account. That only 1 micropylar nucleus should divide and that this division should never have been observed appears contrary to all other observations.

Rutgers (59) has described exactly the same course of development for *Moringa oleifera* as occurs in *Garcinia*, though his account is probably influenced by the authority of Treub. That his claims are also not well founded is apparent not only by various conspicuous shortcomings of his own work but is indicated by the more recent work of Puri (59). It has been shown, for instance, that *Moringa oleifera* follows the normal-type, and there is scarcely any doubt that a re-examination of *Garcinia* would show the same results.

#### OTHER CASES

A few other accounts which in my judgment are subject to question or are founded upon insufficiently accurate data may be mentioned briefly:

1. According to W. R. Smith (66), *Clintonia borealis* follows the *Oenothera*-type. A reinvestigation is needed, since the alleged course of development does not agree with that of closely related plants.

2. *Gastrodia elata*, according to Kusano (45), follows a course of development similar to that described by Pace for *Cypripedium*,

with the difference that the embryo-sac is supposed to develop from only 1 macrospore. For a criticism see Rutgers (1923) and Schnarf (1929, p. 192).

3. *Rudbeckia laciniata*. In this species, according to Palm (53), a 4-nucleate stage without wall formation is produced by the 2 maturation divisions. One macrospore nucleus lies at the micropylar end and from it the micropylar quartette develops by means of 2 successive divisions. Two antipodals develop from the other macrospore nuclei and the third is supposed to form the third antipodal and the lower polar nucleus by division. Confirmation of this form of the *Peperomia*-type is awaited. The author himself regards his work as preliminary.

4. The conditions in *Pandanus* (9, 10, 11) must be regarded as wholly unclarified. To attempt a criticism of the available data would lead only to unreliable and worthless conjectures.

#### CONCLUSIONS

This all too brief discussion, concerned primarily with the most recent findings, indicates that the elucidation and confirmation of data are not yet concluded. The foregoing discussion can only point out certain doubts and omissions in our knowledge and attempt to distinguish in a critical way the established facts from the errors and the doubtful claims. Under such circumstances general conclusions based upon available data can be made only with considerable reservation. Nevertheless, I believe, as stated in my two books on the embryology of angiosperms, that one conclusion may be regarded as fully established concerning the comparative study of embryo-sac development, and this, as has already been indicated, in spite of the by no means unimportant differences in various types of development of the embryo-sac; namely, that the uniformity in development and form of the female gametophyte is so great that it may be regarded as the most important proof of the monophyletic derivation of the angiosperms. The common features among them are, first the development according to the normal-type, and secondly, formation of the embryo-sac from quartettes (*Oangia* according to Chiarugi).<sup>9</sup>

<sup>9</sup> The only important attempt to explain the quartettes phylogenetically is by Porsch (55). His hypothesis that the quartette represents a transformed archegonium is, according to my opinion, in complete harmony with the entire picture which the ontogeny of the female gametophyte among angiosperms presents.

The normal-type may undoubtedly be regarded as the original for the following reasons: of all types it involves the largest number of cell divisions; spore formation and embryo-sac development are separated within it; it is of general occurrence among angiosperms, failing in no group; and finally because from it derivation of all other types is reasonable, whereas it is impossible to regard any other type as the original one from which the normal-type may have been derived.

The derived nature of the abnormal types is evidenced primarily by the fact that they occur for the most part in a great variety of plants. Shortening of the process from the normal- to the *Scilla*-type has appeared in various groups of the Liliaceae, in the Podostemonaceae, in one representative of the Rhamnaceae (*Zizyphus*), in the Boraginaceae (*Anchusa*, *Lycopsis*), in the Compositae (*Erigeron* spp.), among the Alismataceae and the Orchidaceae, in other words, in a great variety of groups among the angiosperms. The same is true also for different forms of the *Peperomia*-type. Phenomena of such distribution can not possibly be regarded as primitive in character. We can hardly regard the *Oenothera*-type, likewise, as primitive, though it is limited to only one family. It appears thoroughly justified, therefore, to regard the normal-type as typical of the angiosperms. The abnormal types are to be looked upon as exceptions which have appeared among various groups and are to be regarded phylogenetically as further retrogressions of the female gametophyte.<sup>10</sup>

A second feature of the female gametophyte, intimately connected with the general concept of the angiosperms is the quartette (Oangium), i.e., a complex of 3 cells and 1 free nucleus. The embryo-sac is a structure consisting of quartettes; the quartette forms the egg apparatus and the antipodal apparatus, and can occur once, twice or four times in the embryo-sac. In the extremely small embryo-sacs of the Podostemonaceae and the Orchidaceae

<sup>10</sup> Shortening of embryo-sac development has occurred also in many parthenogenetic embryo-sacs and the parthenogenetic types corresponding to the normal-type, the *Scilla*-type and the *Lilium*-type have been designated, respectively, as the *Alchemella*-type, *Taraxacum*-type and the *Antennaria*-type. The resemblance between parthenogenetic and normal sexual types is, however, wholly superficial and based upon entirely different causes. Reduction in the parthenogenetic types is founded upon the more or less extensive decline of meiosis, and is a karyological and not a phylogenetic or morphological problem (cf. 29).

it is lacking as infrequently as in those of apomictic plants. This general picture of the occurrence of quartettes is not disturbed by certain exceptions (*Peperomia*, *Plumbagella*). Aside from the fact that any diagnostic feature of a naturally large division can be modified or suppressed in isolated cases, these exceptional cases show, by their relationship to other plants with a typical quartette, that they belong to the angiosperms.

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## EXPLANATORY NOTES

Angiosperms include all flowering plants except the relatively few 600 or so species which constitute the gymnosperms. The former are characterized by the production of ovules which ultimately develop into seeds within an entirely closed and more or less hollow structure, the ovary. Each ovule begins its development as a tiny papilla of meristematic tissue somewhere on the inner wall of the ovary while the latter is still very immature and the two structures develop simultaneously. A single cell in the subepidermal layer of each such papilla is destined to undergo a very special development, all the while remaining embedded in the surrounding tissue. This particular cell is known as the *macrospore-mother-cell* and its further development is the topic of discussion in this paper. The left-hand perpendicular row of circles in Fig. 1 represents this cell in various plants. Each smaller inner circle throughout the figure indicates a nucleus, separated in some cases from adjoining nuclei by a wall, as illustrated.

The macrospore-mother-cell divides usually into 4 macrospores, with or without walls, and in so doing its diploid  $2n$  number of chromosomes is reduced to the haploid  $n$  number in all subsequent nuclei up to fertilization.

One or more of the macrospores enlarge and by repeated nuclear division develop into the female gametophyte, known also as the embryosac, undergoing the changes described in this paper. Some of the ultimate nuclei are then situated at the *micropylar pole*, i.e., at the end of the embryosac nearest the micropyle or cleft between enveloping integuments of the ovule, through which cleft admission of the fertilizing pollen tube is permitted. They there constitute the so-called *egg-apparatus* which consists usually of an *egg-cell* and two additional cells known as *synergids*. Two other nuclei are located in the center of the embryosac and are known as the *polar nuclei* while those at the end farthest from the micropyle are the *antipodals*.

## RECENT DEVELOPMENTS IN FUNGICIDES: SPRAY MATERIALS

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### INTRODUCTION

The word fungicide as used in this paper will follow the definition (91): "Any substance which may be applied to higher living plants in active growth and which will kill parasitic fungi or prevent the development of fungous diseases without killing or seriously injuring the host plant." This definition excludes applications to plants in the dormant stage and to picked fruits.

Since the principal use of fungicides is in the control of diseases of fruits such as the apple, pear, peach, plum, and grape, the principal developments in recent years have been in answer to a demand for better fungicides for these fruits.

Fungicides as here defined are used as protectants (92), that is, "They are usually effective only when applied to the plant before it has become inoculated with the spores of the pathogene." To accomplish this, it is usually necessary to apply the fungicide before the spores are present upon the plant; otherwise, some of them might germinate and infect the plant before the fungicide became effective. Thus the fungicide is a coating over the plant which is noninjurious, or nearly so, to the plant, and at the same time is toxic to spores or germ tubes of certain pathogenic fungi which may come in contact with it.

An ideal fungicide should be toxic to the pathogen, noninjurious or even beneficial to the plant sprayed, even after repeated applications, should cause no toxic accumulations in soils, should be nontoxic to men and animals, cheap and easily obtained, non-explosive, capable of storage without deterioration, noncorrosive, easily made and applied, conveniently handled, capable of covering and sticking well, remaining active over a considerable period of time, and be insecticidal or compatible with the insecticidal sprays without lessening its effectiveness or that of the insecticide.

The common fungicides at present in use possess most of these characters to some degree, but not to the degree desired. For

instance, a spray which is sufficiently toxic to prevent infection may be unsafe to use because of the risk of severe injury to the sprayed plant. A realization of the properties which a good fungicide must have will show at once why the development of new fungicides is a hard task. When one realizes that he is applying a spray to a plant to protect it from another organism which is also a plant, he must realize that the balance between noninjuriousness to the sprayed plant and toxicity to the pathogen is very delicate indeed. Also the fact that fungicides which are sprayed upon trees in large amounts, perhaps 60 gallons per tree in one growing season, must be cheap is very discouraging to those attempting to develop new fungicides.

In recent years much work has been done to improve the older fungicides and develop new ones. Attempts have been made to find out how the various chemicals or combinations of chemicals act on fungous spores and on leaves, fruits, and other parts of the sprayed plant; also the effect of weathering on the composition, toxicity, and durability of the dried residues remaining after the fungicides have been applied.

Because the mechanism by which a fungicide kills spores appears identical with that by which it may injure the plant to which it is applied, it is evident that susceptibility to the working of this mechanism on the part of the fungus spore and resistance on the part of the sprayed plant are greatly to be desired. More efficient fungicides can be applied to injury-resistant species or varieties than to those susceptible to spray injury. On the other hand, the less effective fungicides will often control diseases without injury on species or varieties resistant to the diseases but susceptible to injury by the more effective fungicides, especially if the spores of the pathogen are easily killed. Resistance to spray injury and resistance to diseases often, therefore, influence the choice of a fungicide and lessen the risk (1) of failure to control diseases and (2) of serious damage from the spray itself.

The mechanism by which fungicides are largely prevented from entering the cells of the sprayed plant and consequently injuring them has not received the attention which it deserves. It is generally conceded that the entrance is prevented by the cuticle; but the cuticle, as an organ which largely prevents spray injury, has received little attention. It would be interesting to know how

readily the cuticle can be ruptured and whether or not wind, rain, hail, and the mechanical effect of spraying produce microscopically small ruptures through which sprays may gain access to and kill the underlying tissues. It would also be desirable to know the physical and chemical effects of the various fungicides and insecticides on the cuticle and epidermis, and the influence of weather, soils, fertilizers, pruning, and other cultural practices on the structure and development of these organs.

The problem of safe and effective fungicides for species and varieties injured by the fungicides now in use is a hard one to solve, and some of its most important aspects are strictly botanical.

Fungicides may be divided into three classes, according to their essential ingredients: (1) sulphur, (2) copper, and (3) those containing neither sulphur nor copper as essential ingredients.

#### SULPHUR

Lime-sulphur (calcium polysulphide) solution, for a quarter of a century the standard spray for the control of apple-scab, often causes more injury to fruit and foliage than is desired. Many attempts have been made to modify this solution so as to lessen the danger of injury without materially reducing its fungicidal efficiency. Most of the suggested modifications consist in adding to the lime-sulphur solution chemicals having an acidic reaction, so that part or all of the polysulphide sulphur is precipitated as elemental sulphur (1, 3, 29, 37, 41, 49, 50, 51, 73). These modified lime-sulphurs appear not to have any particular advantage over a weaker solution of lime-sulphur or one of the finely divided elemental-sulphur sprays already obtainable, or a mixture of the two.

Self-boiled lime-sulphur, containing elemental sulphur as its active ingredient and the first successful fungicide for the control of peach brown-rot (*Sclerotinia fructicola*), has been almost wholly superseded by sprays in which very finely divided sulphur is held in suspension in water. Some of these sprays have been used successfully in the control of apple-scab (*Venturia inaequalis*) in place of the fungicidally more efficient but more injurious lime-sulphur solution (3, 5, 19, 29, 30, 37, 64, 89). These so-called wettable sulphurs are obtained or prepared in various ways, of which the following are examples: As a by-product in the manu-

facture of illuminating gas (89); by mixing or grinding very finely ground sulphur with a colloid such as casein or glue (84); by fusing the sulphur with a colloidal clay such as bentonite and grinding the product to an extreme fineness (30, 37); by precipitating sulphur from lime-sulphur solution (101); and by the interaction of sulphur dioxide and hydrogen sulphide (90).

Sulphur in dry form, to be applied as a dust, has been improved in recent years by finer grinding and the addition of wetting agents (94). It is used extensively on peaches for the control of brown-rot and scab, *Cladosporium carpophilum*.

The question as to how a chemical as insoluble as elemental sulphur acts as a killer of fungus spores has evoked many different answers. Where formerly it was assumed that such substances as sulphur dioxide or sulphuric acid were formed which act directly on the spores, recent investigations have failed to support these assumptions; but the investigators do not agree among themselves as to what the toxic substance is. Certain investigators produce evidence to show that pentathionic acid is formed as the toxic principle (22, 52, 53). This is disputed by others who point out that sulphur mixed with alkaline substances such as lime, which would neutralize pentathionic acid, remain toxic (86, 93). Some investigators hold to the view that the most important toxic substance is hydrogen sulphide, to which they maintain sulphur is reduced when in contact with spores or leaves (9, 22, 57, 58, 66). It is supposed that some substance, a chemical or an enzyme, produced by plants is capable of reducing sulphur to hydrogen sulphide. There is some evidence that this substance may be glutathione (9, 66). Some consider sulphur itself to be toxic (31, 86). It has been shown that the polysulphides of lime-sulphur solution may be directly absorbed by spores with the deposition of elemental sulphur (32). Since many of the derivatives of sulphur have been shown to be toxic to fungus spores, it is probable that under different conditions different compounds may be formed which act as effective fungicides. This view is further supported by the fact that the sulphur fungicides are effective with or without lime and under all combinations of weather.

Lime-sulphur solution is less apt to burn if applied at a time when it will dry quickly. Quick drying reduces the time in which

the original solution, gradually changing over to less injurious substances, chiefly elemental sulphur, is able to act with its full strength. The elemental-sulphur sprays and dusts do not cause the type of burning that lime-sulphur may cause when first applied, but under conditions of high temperatures and bright sunshine all types of sulphur sprays may burn.

Even when sulphur sprays do not burn they may cause leaves to turn yellow and fall or an entire plant to cease growth and take on a yellow, sickly appearance. For this reason the sulphur sprays are little used on potatoes, beans, grapes, and many other plants. Lime-sulphur sprays may decrease photosynthetic activities of apple leaves with an accompanying decrease of sugar content in the ripe fruit (23, 42, 43, 44).

#### COPPER

Bordeaux mixture remains the standard and most used copper fungicide, as it has for the past half century, but by the so-called "instant" process the home-made mixture is more quickly and more easily made than formerly (83, 84, 85). In this process, granulated copper sulphate is dissolved directly in water in the spray tank and then hydrated lime is added. The mixture should be stirred vigorously throughout the process and during use. Factory-made bordeaux mixture is physically much better than formerly and contains a much higher percentage of copper. Bordeaux mixture frequently injures certain plants severely, such as peaches and apples, but even when there is no direct injury its use is sometimes undesirable during periods of drought, because, at least with certain plants, it increases the transpiration rate (97, 98, 99, 100). There is some evidence that this increase may be prevented by the addition of a small quantity of petroleum oil emulsion (97). However, for apple and pear leaves sprayed with copper fungicides, slower wilting and a lessened transpiration rate have been reported (69). A bordeaux mixture in which magnesium lime was used has given better results on potatoes (10), and has increased transpiration somewhat less than bordeaux mixtures in which high calcium lime was used (98). Varieties of apples and pears, the leaves of which have high osmotic values, are said to be less sensitive to copper injuries than those with leaves having low osmotic values (69). Excepting bordeaux mixture, the older

copper sprays, such as burgundy mixture and ammoniacal copper carbonate, are little used, principally because of their injuriousness to foliage. A dust composed of copper sulphate monohydrate and hydrated lime is still used, especially on truck crops, and has been changed little in recent years.

Despite the shortcomings mentioned above, copper sprays have certain advantages over the sulphur fungicides, their principal rivals for popularity. Copper is generally a better killer of fungus spores (the powdery mildews are a notable exception to this) and when it does not directly injure, it usually has no unfavorable effect on the plant sprayed. Sulphur and sulphur compounds, on the other hand, frequently cause a yellowing and stunting, even if they do not cause direct burning.

Much experimental work is now in progress to develop copper sprays less injurious than bordeaux mixture and sulphur sprays without materially reducing fungicidal properties. Because bordeaux mixture has been shown to contain considerable amounts of soluble copper (11, 12, 13, 15, 39), most of these experiments are based on the assumption that the so-called "insoluble" copper compounds will not cause serious injury to the sprayed plant, but in contact with spores will become sufficiently soluble to be either toxic to spores or to prevent germination. These "insoluble" copper materials are usually mixed with colloidal clays, colloidal clays and lime, or organic substances to keep them in suspension in water and to increase their spreading or sticking properties. The following copper compounds, most of which belong to the "insoluble coppers," have been subjected to recent experimentation and, in some cases at least, are being improved by the use of more finely divided materials and better stickers and spreaders: copper silicate (33), copper ammonium silicate (4, 37, 64, 77, 88, 102), red copper oxide (37, 38, 54, 77), copper phosphate (33, 82, 102), basic copper sulphate (29, 45, 102), copper oxychloride (102), copper sulphide (27, 28, 73), black copper oxide (33), copper resinate (24), copper zeolite (4), and Raleigh's mixture, containing copper sulphate, lye and molasses (78).

Recent work has confirmed an older idea that toxicity of copper sprays depends upon soluble copper (14, 15, 21, 34), which, if not already present in the spray in a lethal concentration, may be brought into existence by the action of spores upon the insoluble



copper of the spray residues (65, 79). It has also been shown that spores may bring about their own death by the rapid absorption of copper from "insoluble" copper spray residues (34, 79, 80). Recent work has indicated that fungus spores produce malic and possibly other acids which are capable of bringing into solution toxic quantities of copper from dried bordeaux mixture residues (68).

#### OTHER FUNGICIDES

Sprays other than those containing copper or lime as essential ingredients have been extensively investigated, but only a very few have proved successful. Sodium carbonate (washing soda) has been recommended for the control of American mildew (*Sphaerotheca mors-uvæ*) of gooseberries (71). Potassium permanganate is sometimes used to arrest the growth of powdery mildews, but since its action is quickly over, it must be followed by a sulphur or copper spray. Aluminum salts have been tested and sometimes recommended, but they show little promise of becoming useful fungicides (56). Alum alone or with bordeaux mixture has controlled grape downy mildew (*Plasmopara viticola*) (87). Aluminum sulphate has been added to lime-sulphur solution for the purpose of precipitating the sulphur from the calcium polysulphides, and the resulting mixture has been applied as a spray, but the aluminum probably adds little or nothing to the fungicidal properties of the mixture (1, 29, 41, 51).

Calcium is an important ingredient of the principal fungicidal sprays, lime-sulphur (calcium polysulphide) and bordeaux mixture, although it is only weakly fungicidal. It is, however, very useful in the form of milk of lime (calcium hydroxide), chiefly in the prevention of injury to sprayed plants. The lime, until it becomes completely carbonated, neutralizes the acidic substances formed in copper, sulphur, and arsenic spray residues, rendering them less harmful to the plant.

Barium sulphide formerly was used to some extent, but is now little used. It is inferior to calcium polysulphide as a fungicide (35).

Selenium compounds, because of their close chemical relationship with sulphur compounds, have received some attention, but are more injurious to plants than the sulphur compounds and

possess no advantages otherwise (95). Results of recent investigations on the effect of selenium-bearing soils on plants and on animals eating the plants would prevent the use of selenium compounds even if they were good fungicides (20, 72).

Ferrous sulphate has been used to a limited extent as a fungicide, but is generally unsatisfactory. It is more often used as a spray to correct a chlorotic condition caused by deficiency of available iron rather than as a fungicide. When added to lime-sulphur solution, ferrous sulphate produces a mixture containing ferrous sulphide and sulphur which has been used to some extent for the control of apple powdery mildew (8) and apple scab (1, 3, 50, 51, 73).

Many compounds of mercury are toxic to fungus spores even in dilute solutions, but in their present development they are too injurious to the sprayed plant to be useful as fungicides. Mercuric chloride diluted 1 part to 1000 parts of water is highly toxic to fungus spores, but is very injurious to vegetation and possesses no resistance to weathering. Silver compounds also are very toxic to fungus spores, but their cost would be prohibitive even if they could be shown to be desirable otherwise (67).

Zinc-lime, made by combining a solution of zinc sulphate with milk of lime, is used on peaches for the control of bacterial spot (*Bacterium pruni*) (81) and the prevention of arsenical spray injury (46, 48, 76, 81). It is not only noninjurious to peach trees but has a favorable effect on certain types of chlorosis. It is a weak fungicide, however, and cannot be depended upon to control scab and brown-rot. It is also used as a "corrective" spray for certain nonparasitic diseases of plants such as apple and pecan rosette, bronzing of tung-oil-tree leaves, mottle-leaf of citrus, and "little-leaf" of various fruit plants (18), when there is danger of burning from the use of zinc sulphate alone.

Studies of the elements and their compounds have shown that compounds of osmium, cerium, cadmium, lead, and thallium are toxic to fungi, but none of these is at present listed as a promising fungicide (67).

Of the vast list of organic materials, many of which are so useful as germicides, none can at present be considered as a practical fungicide. Soaps have some use in the control of powdery mildews, but are inferior to sulphur. Many of the dyes

are toxic to spores but do not otherwise possess the properties of fungicidal sprays (17, 70, 75).

Mineral oils have only slight fungicidal properties, and at their most effective strengths are apt to cause injury (61).

The tar oils at effective strengths are very injurious to foliage. (61).

The vegetable oils are weak fungicides (7, 61) but as a class are apparently less apt to cause injury than either the mineral oils or tar oils. They appear promising at the present time, not as fungicides but as spreaders to be added to fungicidal mixtures.

A wide range of manufactured hydrocarbons and their simpler hydroxyl derivatives and esters have been tested in a small way and in the form of emulsions for the control of hop mildew (*Sphaerotheca humuli*) (62). "Benzene, cyclohexane, dekaline, cymene, carvene, phellandrene, dipentene, turpentine, pine oil, geraniol, eucalyptus oil, and fenchone were phytocidal (i.e., injurious to the leaf in areas not invaded by the fungus) at the lowest concentrations at which they were fungicidal.  $\alpha$ - and  $\beta$ -naphthol were fungicidal at concentrations of .15 and .2 percent., respectively, and, except in one experiment, were not phytocidal at concentrations under .5 percent. As similar results were obtained with commercial grades of  $\alpha$ - and  $\beta$ -naphthol, these appear to merit further trial for the control of powdery mildews. The polyhydric phenols and the phenolic acids tested were fungicidal only at concentrations at which serious leaf injury was caused. Saligenin, salicylaldehyde, and vanillin were inactive at concentrations of about 1 percent., while paranitrophenol and picric acid were strongly phytocidal. Salicylanilide, applied in the form of its sodium salt. . . . was fungicidal at a concentration of .5 percent., almost fungicidal at one of .25 percent., and not injurious to the leaf at one of 1 percent. Suspensions containing 1 percent salicylanilide were not fungicidal, but were more active when soap was used as the spreader. . . . None of the esters tested proved likely to be of practical value as a fungicide." (From abstract in Rev. Appl. Mycol. 13: 790. 1934). Also the fungicidal action of organic thiocyanates, resorcinol derivatives, and other selected organic compounds have been tested in the laboratory (96). Benzoic acid with a linseed-oil spreader has given good results as a spray for the control of downy mildew of tobacco (*Peronospora*

*tabacina*). Picric acid was less effective and injured the foliage (38).

While it is probably true that the fungicide of the future will be of organic nature, a great deal of work will need to be done before one is developed. At the present time none of the organic compounds or their derivatives shows promise of taking the place of the standard inorganic fungicides now in use.

#### SPREADERS AND STICKERS

Many substances have been suggested for addition to fungicides to cause them to spread out or "crawl" over the surface of leaves and fruits or to adhere for a longer time. Most of these substances are of the "spreader" type and when added to fungicides cause the film of air in contact with the plant to be displaced and the droplet of spray to flatten out.

Theoretically, spreaders should be very desirable, but in actual practice they are frequently disappointing because they may leave too thin a film over the plant to give adequate protection against fungi and they may lessen the effectiveness of the fungicide (16, 55, 58), presumably by coating its particles, or reacting with it to form nontoxic compounds.

The principal substances under test are soap, glue, gelatine, casein, bentonite and other clays, bentonite-lime, the lighter mineral oils, fish oils, vegetable oils, waste sulphite liquor from paper mills, soluble resins, sulphates of the higher alcohols, salts of alkylated aryl compounds, and various other organic substances. (1, 2, 3, 6, 16, 25, 26, 29, 36, 40, 47, 49, 50, 55, 59, 60, 63, 73, 74, 77). The addition of spreaders to two of the most used fungicides, bordeaux mixture and lime-sulphur solution, which already have good spreading and adhesive qualities, has not materially increased their effectiveness, and may decrease it by lessening the thickness of the residue. The usefulness of spreaders and stickers is also limited by the fact that plants in an active growing condition must be sprayed frequently to protect the new growth, even if the residue from previous applications is still intact.

For adding wetting and spreading properties to the elemental sulphur sprays, casein, glue, bentonite, soap, and other substances have proved their value. They are essential ingredients of commonly used sulphur sprays. Spreaders and stickers may prove

useful in the development of sprays such as the "insoluble" coppers, which do not possess the spreading and adhering properties of bordeaux mixture (33, 54, 77, 82, 102).

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